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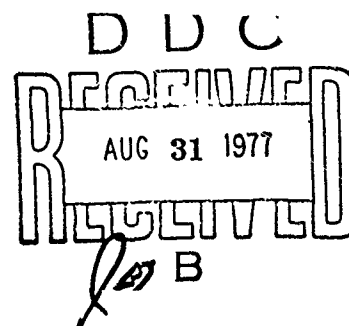
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U.S. ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

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This report documents the clinical and laboratory activities of the US Army Institute of Surgical Research during fiscal year 1976. These activities include patient care, clinical investigation and laboratory research in the areas of (1) burn injury, (2) acute renal failure, and (3) general trauma. Special emphasis is placed on the clinical management of burned patients and on studies related to prevention and treatment of burned wound infection.		

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FOREWORD

The effectiveness of categorical health care in terms of clinical results and research productivity is well illustrated by the material within this volume produced by the concerted effort of enthusiastic, hard-working, and generally optimistic laboratory and clinical staff. Not only has average duration of hospital stay for our burn patients decreased over the past five years but improved functional results have also been achieved in many patients with severe burns, and clinical and laboratory investigations have led to improved diagnosis of inhalation injury, effective prophylaxis against Curling's ulcer, identification of basic defects in host resistance, and improved techniques of metabolic and nutritional support. This clinical-laboratory symbiosis, which has broad medical applicability, is dependent upon several factors which are in themselves mutually reinforcing.

The Army Medical Department has, by concentrating burn care at this Institute, ensured an adequate and dependable density of patients. The Medical Research and Development Command, by farsighted personnel management, has provided for stability of senior staff, assignment of selected clinical staff and the emplacement of a multidisciplinary research team. Planned construction by the Medical Department has also established sophisticated research laboratory facilities. The staff, in turn, has identified significant problems for study, organized interdisciplinary research teams, established dedicated clinical study areas, promoted active involvement of allied scientists and, as noted above, applied research techniques in an innovative fashion to the successful resolution of clinical problems. This symbiosis, which must exist for effective, clinically relevant research, benefits the individual staff member by facilitating his scientific and clinical productivity and benefits the Army by the merit of the end product and the productivity of the Institute. Most importantly, the burned or otherwise injured soldier remains the focal point of all the care and research activities of the Institute and benefits from these combined individual and organizational efforts.



BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander & Director

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<p>23. (U) The Clinical Division of the US Army Institute of Surgical Research continues its role as a major specialized clinical treatment center for thermally injured military personnel. Its main objectives are the investigation and modification of new diagnostic and therapeutic methods for optimum care of the burn patient as well as dissemination of the scientific advances to military and civilian medical treatment centers.</p> <p>24. (U) Thermally injured patients both from the Continental United States and throughout the world are evacuated to the US Army Institute of Surgical Research for intensive inpatient therapy. Carefully controlled clinical evaluation of the efficacy of many treatment modalities is undertaken.</p> <p>25. (U) 75 01 - 75 12 During 1975, 254 burn patients were admitted to the Institute of Surgical Research. Specific attention to the early diagnosis and treatment of inhalation injury involved the use of fiberoptic bronchoscope and xenon scan for the diagnosis and treatment of inhalation injuries. In a double-blind study steroids in inhalation injuries were found to be non efficacious. Early fluid resuscitation was evaluated with ongoing attention to the role of colloid in the first 24 hours post burn. As in previous years, pulmonary infection with gram-negative organisms continues to be a frequently observed complication of thermal injury and intensive investigation in methods of prevention and treatment continues. Continued evaluation of the role of topical chemotherapeutic agents is proceeding with an attempt to delineate the true role of topical therapy in the care of the large thermal injury. General principles of management previously described at this unit remain basically unchanged. Several new clinical approaches to the treatment of the extensive thermal injury and its complications are presently being evaluated. These include earlier excision both formal and tangential, maintenance of an environment more tolerable to the patient, i.e., warmer and more rigorous pursuit of the patient's nutritional requirements.</p>							

ANNUAL PROGRESS REPORT

PROJECT NO. ~~3461102B71R-01~~, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1975 - 31 December 1975

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 January - 31 December 1975

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Two hundred and fifty-four patients with thermal injury were admitted to the Clinical Division of the United States Army Institute of Surgical Research during the calendar year of 1975. The Institute's main emphasis continues to be providing optimal care to military personnel and authorized civilians with major thermal injuries. Clinical investigation has continued into the physiology, biochemical and bacteriologic aspects of thermal injury. Continued evaluation of the role of topical chemotherapeutic agents is proceeding with an attempt to delineate the true role of topical therapy in the care of patients with extensive thermal injury. Several new clinical approaches to the treatment of the extensive thermal injury and its complications are presently being evaluated. These include earlier excision both tangential and at the level of the investing fascia, maintenance of a warm microenvironment to decrease energy needs, and improved nutritional support. This report summarizes the activity of the Clinical Division of the United States Army Institute of Surgical Research in 1975 and cites the recognizable complications which have contributed to mortality and morbidity in burn patients.

Thermal injury
Resuscitation
Air evacuation
Topical therapy

Heterograft
Autograft
Homograft
Inhalation injury

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division of the United States Army Institute of Surgical Research continued through the year of 1975 to have as its primary objective the provision of clinical care for the thermally injured soldier. The number of admissions increased from 244 in 1974 to 245 patients in 1975.

In 1975 there were a total of 87 aeromedical evacuation flights. 86 of these were CONUS flights (within the Continental United States) with 108 patients evacuated. All patients within a radius of 200 miles of Brooke Army Medical Center requiring air evacuation were transported by helicopter. There were 18 flights for that purpose. There was one flight outside of the Continental United States that being to Alaska.

CLINICAL MANAGEMENT

In depth description of the management of patients with thermal injury as practiced by this Institute are found in previous Annual Reports and in numerous scientific publications. Therefore the following remarks will be limited to new and current methods of clinical therapy.

1975 was the first year in which silver sulfadiazine was used exclusively as the initial primary topical chemotherapeutic agent. The initial success noted during the period of comparison treatment with Sulfamylon in 1974 did not continue to the degree first noted. During 1975 there was a remarkable increase in the resistance of the *Klebsiella* cultured from the wounds to silver sulfadiazine. A complete change of the predominant organism in the burn wound from *Enterobacter cloacae* to *Klebsiella* was seen. This increased resistance was heralded by earlier time of positive blood culture, earlier onset of sepsis and earlier time to death in the large burns. There has also been a decrease in the LOSO in the 15 to 40 age group so that it is now less than the 63.5 noted in 1974. The premises of the earlier comparison study were again born out through 1975 that being that Sulfamylon offers a far better bacterial control of the gram negative flora of the burn wound, but may exert deleterious systemic effects on patients with burns greater than 30% in the early post burn period.

Early pulmonary complications characterized by hyperventilation, respiratory alkalosis with progression to a fluid overload pattern and the appearance of "shock lung" were rare in 1975. The major pulmonary complications in 1975 were fluid overload secondary to vigorous resuscitation which responded rapidly to diuresis and fluid restriction and pneumonitis secondary to both inhalation injury and gram negative infection per se. The lack of significant early pulmonary complications except those related to severe inhalation injury bespeaks the benign properties of silver sulfadiazine as an initial topical agent, however, during 1975 this positive quality began to be outweighed by silver sulfa-

diazine's lack of efficacy against the gram negative population of the burn wound and a re emergence of Pseudomonas organisms in the burn wound of terminal patients. However it remains our opinion that Sulfamylon does offer better bacteriologic control of the burn wound but it may cause marked hyperventilation in patients with burns greater than 30% and it is our opinion that it should be reserved for use as the topical agent for treatment of heavily colonized or infected burn wounds.

The ¹³³Xenon lung scan, fiberoptic bronchoscopy, and pulmonary function testing continued to be used for the documentation of inhalation injury. As has been noted in the past we have obtained falsely positive xenon scans due to chronic lung disease or acute upper respiratory infection which existed prior to burning. However, when the ¹³³Xenon lung scan is correlated with bronchoscopy falsely positive diagnoses are rare. At present we are evaluating nebulized gentamicin in a double blinded fashion in patients with positive findings of inhalation injury. To date no results are available. At present we administer large doses of steroids systemically and aerosolized racemic epinephrine in the treatment of upper airway inflammation to reduce or limit edema formation and avoid intubation. Attempts are being made to correlate pulmonary function with the status of the acute upper airway before and after such treatment in an effort to document its efficacy.

In 1975 as in previous years the two major fluid and electrolyte problems seen at the Institute of Surgical Research were hypernatremia and hyponatremia. Hypernatremia reflecting a water deficit was found to be the most common electrolyte abnormality in the burn patient. Hyponatremia is the most common electrolyte change in the burned child and is often manifested by seizures. It is caused either by excessive administration or too rapid administration of electrolyte free fluids or the "leeching" effect of silver nitrate dressings when used. These findings again call for caution in the administration of nonelectrolyte fluid in small children and for the careful monitoring of all patients in silver nitrate dressings and for careful observation of all patient's electrolyte status during resuscitation.

Interest has continued during 1975 in primary excision both to the level of the investing fascia and tangential. Twenty-one formal excisions to the level of the investing fascia were done while 42 tangential excisions were carried out in 1975, 23 of these being excision of burns of the hand. The excisions to the level of the investing fascia fell into two categories; excisions of massive burns with immediate coverage with cutaneous allograft or xenograft, all of which have done poorly and excision of localized full thickness injury in patients with moderate sized burns 40-60%, all of which have done well in 1975. Evaluation of tangential excision continued throughout 1975. Tangential excisions also fall into two groups. The first being tangential excision of burns of the hands with immediate autografting. This is carried out on deep second degree burns of the hand that have no significant areas of third degree involvement. The functional results have been excellent and this treatment has much to recommend it over scalpel excision of the hands.

When the hands that were tangentially excised had deep second degree burns, the results were uniformly excellent. In the few cases where full thickness injury was recognized and excised, the results were indistinguishable from classical conservative treatment. Tangential excision was also employed in patients having full thickness wounds with tenacious eschar. The eschar was tangentially excised until freely bleeding yellow fat was encountered. These areas were either covered with biologic dressing or with Sulfamylon soaks and allowed to granulate. The wound matured and good granulation tissue formed in seven to 14 days permitting subsequent "mesh" grafting.

Interest has continued in the metabolic status of the burn patient during 1975. Glucose flow through the extravascular compartment has been evaluated and found to be significantly elevated in 17 burn patients between the 6th and the 16th post burn day when compared to normal individuals. This shows an increased rate of gluconeogenesis in the second post burn week that was directly related to the extent of injury and with time returned in a curvilinear manner towards normal with closure of the burn wound. In contrast to this glucose flow decreased towards normal in the patients with gram negative infection. The elevated glucose flow in patients with extensive burn injuries drops precipitously towards normal with onset of gram negative sepsis. Simultaneous measurements of glucose flow and oxygen consumption demonstrated a close relationship between gluconeogenesis and heat production.

Glucagon administration in normal man was also evaluated. Increased heat production occurred with glucagon administration in a hypothermic elderly burn patient. This increased heat response is closely related to and appears concomitantly with translocation of glucose to the periphery, but cannot be duplicated by glucose infusion alone. The increased caloric needs of the burn patient appear to reflect the increased energy requirements of accelerated gluconeogenesis.

During 1975 intragastric instillation of antacids was used as prophylaxis against the complications of Curling's ulcers. Sufficient antacid was instilled on a q i to 2 hour basis to maintain the pH of the stomach contents at 7.0 or above. This has been attended by a marked decrease in clinical complications of stress ulcers so that only one major GI bleeder required operation in 1975. We have made antacid prophylaxis a routine in the early post burn course with excellent results.

EDUCATION

During the period of this report, 2 surgical residents from Brooke Army Medical Center, 1 from Fitzsimons, 3 from David Grant USAF Medical Center, Travis AFB, California and 1 civilian physician from Northwestern and 1 resident from the University of Wisconsin at Milwaukee participated as active members of the medical staff for periods of 2 months to 1 year as part of their surgical training. A physician from the Navy of Sweden,

two Army and one Navy reservist completed their tours of active duty training with our unit and one senior medical student from the University of Texas in San Antonio, one from Northwestern, and one from the University of Missouri School of Medicine spent from two weeks to one month in observer training. Twenty-five Air National Guard officers and paramedical personnel from one unit were given a two hour tour and briefing. Approximately 93 civilian and military physicians and 130 nurses, students and paramedical personnel visited and were briefed during 1975. Forty-two foreign visitors from the following countries: Belgium, Japan, France, Norway, Ireland, Israel, Sweden, England, Switzerland, Germany, Denmark, Korea, Republic of Zaire, Holland, Uruguay and the Republic of Vietnam, received briefings on the overall mission of the Institute of Surgical Research and the care of the thermally injured patient.

Numerous scientific presentations concerning various aspects of thermal injury were made by members of the Clinical Division at local, state, regional and national meetings as listed at the end of this section.

STATISTICAL RESUME

During the year 1975, 245 thermally injured patients were admitted to the Institute of Surgical Research. There were 254 dispositions during 1975 and the subsequent data will be based upon those dispositions. The patients ranged in age from 6 weeks to 85 years with 208 males and 46 females. The average age of the patient was 29 years with an average burn size of 42.1% and a 17.9% average third degree component. The average burn index was 29.7. Out of 254 dispositions, 197 had third degree burns. Forty patients were less than 15 years of age with an average age of 4.75 years. The average total burn size in the pediatric age group was 32.2% with a 14.2% third degree. The burn index in children was 22.5. Of the 40 pediatric patients admitted, 24 had some third degree burn (60%).

The mortality in the pediatric age group was 20%. In the group of pediatric burn patients who died, the average age was 5.5 years and the average burn size was 64.8% with 44.6% third degree injury. The overall mortality for the year 1975 was 37% or 94 patients out of the 254 dispositions. Of the patients expiring 73 were male and 21 were female. The average age of the patients who died was 31.8 years, the average total burn size was 61.3% with 32.8% being third degree involvement, with an average burn index of 46.3%. There was a 6% decrease in raw mortality but the mortality in general remains higher than during the period when many patients were air evacuated from Southeast Asia. Of the 94 patients who expired 89 or 94.9% had some areas of third degree burn. Autopsies were performed in 72 patients or 76.6% of all deaths. The average post burn day of death was 16.6 which was decreased from the 21.4 mean time to death in 1974 but still significantly longer than the 11.8 days in 1973. Table 1 identifies the source of admission of patients during calendar year 1975. The majority of the patients were

Table 1. Source of Admission, 1975

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	4	0	1	0	0	0	5	4	14
3rd Army	5	5	3	3	7	2	7	14	46
5th Army	18	11	9	9	7	2	10	63	135
6th Army	2	0	1	3	2	2	5	8	23
MDW	1	0	0	0	0	0	0	0	1
Alaska	0	0	1	0	0	0	0	1	2
Germany	5	3	2	0	1	0	0	0	11
Ecuador	0	0	0	0	0	0	0	2	2
Hawaii	0	0	0	0	0	0	0	1	1
Mexico	0	0	0	1	0	0	0	4	5
San Salvador	0	1	0	0	0	0	0	0	1
Okinawa	1	1	1	0	1	0	0	0	1
Korea	1	0	0	0	0	0	0	0	1
Costa Rico	0	0	0	0	0	0	0	1	1
Panama	0	1	0	0	0	0	0	1	2
Honduras	0	0	0	0	0	0	0	4	4
Virgin Island	0	0	0	0	0	0	0	1	1
	37	22	18	16	18	6	33	104	254

A - Army

AF - Air Force

D - Dependent

Other: Civilian Emergency

US Public Health Service Beneficiary

Bureau of Employees Compensation Beneficiary

N - Navy, Marine Corps & US Coast Guard

VAB - Veterans Administration Beneficiary

Table 2. Burn Etiology, 1975 - 254 Dispositions

Causes	Number of Patients	% Disposition		Deaths	% Mortality	
Gasoline & Kerosene	60	23.6%		22	36.7%	
Structural Fires	26	10.2%		16	61.5%	
Motor Vehicle Accidents	15	5.9%		2	13.3%	
Aircraft Accidents	11	4.3%		6	54.5%	
Open Flames	23	9.0%		8	34.8%	
Electrical	14	5.5%		1	7.1%	
Hot Liquid	21	8.3%		1	4.8%	
Chemical	2	0.8%		1	50.0%	
Others	17	6.7%		7	41.2%	
Bucalite, Propane or Natural Gas Exp.	35	13.8%		15	42.9%	
Welding Accidents	9	3.5%		1	11.1%	
Smoking Cigarettes Ignited	17	6.7%		14	82.4%	
Mortar Shell Exp.	4	1.6%		0	0.0%	
TOTAL	254			94		

Table 3. Age, Body Surface Involvement & Mortality, 1975

Age (Yrs)	Per Cent Burn										Total		Mortality
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Cases	Deaths	
0-1	1	1	1	1	1	1(1)	0	0	0	0	6	1	16.7
1-2	2	3	1	0	1(1)	0	0	0	0	0	7	1	14.3
2-3	0	3	1	2	1	0	1(1)	9	0	0	8	1	12.5
3-4	0	0	0	0	0	0	0	1(1)	0	0	1	1	100.0
4-5	1	2	0	0	0	0	0	0	0	0	3	0	0
5-10	0	2	1	1	0	1	1(1)	0	0	1(1)	7	2	28.6
10-15	1	0	2	2(1)	0	1	1	0	1(1)	0	8	2	25.0
15-20	4	6	2	1	4(1)	1	5(4)	2(1)	0	2(2)	27	8	29.6
20-30	3	12	9	13(3)	8	13(5)	17(10)	7(5)	7(6)	0	89	29	32.6
30-40	5	4	2	7	3(1)	2(2)	2(1)	1(1)	0	1(1)	27	6	22.2
40-50	3	1	3	7(4)	4(1)	7(5)	3(2)	4(4)	1(1)	0	33	17	51.5
50-60	0	0	3(3)	6(1)	4(2)	2(1)	1(1)	2(2)	1(1)	1(1)	20	12	60.0
60-70	0	0	2(1)	2(2)	1(1)	0	3(3)	1(1)	2(2)	0	11	10	90.9
70-80	0	2(1)	1	0	1(1)	0	0	0	1(1)	0	5	3	60.0
80-90	0	0	1	0	0	0	0	1(1)	0	0	2	1	50.0
Total	24	36	29	42	28	28	34	19	13	5	254		
Deaths	0	1	4	11	8	14	23	16	12	5		94	
% Mortality	0	2.8	13.8	26	28.6	50	67.6	84.2	92.3	100			37.8

Note: Deaths shown in parentheses.

Table 4. Per Cent Body Surface Involvement and Mortality, 1972 - 1975

% Burn	0-14	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
(1972)											
No. Burned	47	56	43	42	36	23	22	16	11	5	301
Deaths	1	2	7	13	15	13	21	15	11	5	103
% Mortality	2.1	3.6	16.3	31	41.7	56.5	95.4	93.8	100	100	34.2
(1973)											
No. Burned	39	35	46	26	38	32	20	16	12	9	273
Deaths	0	0	7	9	21	22	17	16	12	9	113
% Mortality	0	0	15.2	34.6	55.3	68.8	85	100	100	100	41.4
(1974)											
No. Burned	26	24	28	32	36	28	19	10	14	9	226
Deaths	0	0	4	10	18	17	17	9	13	9	97
% Mortality	0	0	14.3	31.2	50	60.7	89.5	90	92.9	100	42.9
(1975)											
No. Burned	20	36	29	42	28	28	34	19	13	5	254
Deaths	0	1	4	11	8	14	23	16	12	5	94
% Mortality	0	2.8	13.8	26.2	28.6	50	67.6	84.2	92.3	100	37

Table 5. Per Cent Burn Versus Survival, 1955-1975

Year	Survivors (burns over 30%)			Deaths		
	No. Cases	Average % Burn Total	%	No. Cases	Average % Burn Total	%
1955	20	39.5	20.3	21	55.6	38.1
1956	22	41.0	17.3	26	57.8	37.8
1957	19	38.4	24.1	17	57.1	38.8
1958	15	42.3	21.6	23	56.5	35.3
1959	29	43.1	20.6	24	63.1	38.1
1960	17	44.2	20.1	30	57.8	37.3
1961	18	44.2	25.0	37	58.0	39.7
1962	18	42.7	21.4	54	59.1	46.2
1963	28	45.8	19.6	57	69.3	41.0
1964	40	41.8	14.8	37	65.0	42.4
1965	47	43.8	21.0	33	66.0	33.4
1966	68	41.5	14.9	59	59.9	31.3
1967	103	42.7	13.3	51	59.9	32.3
1968	143	44.2	12.6	38	54.6	24.6
1969	113	43.2	11.1	70	58.7	26.4
1970	92	39.4	10.7	70	51.9	32.6
1971	63	41.9	14.0	68	60.8	38.0
1972	62	42.0	17.2	103	56.7	35.9
1973	47	43.7	19.6	113	60.3	36.2
1974	55	43.9	12.2	97	60.8	35.5
1975	80	46.1	14.7	94	61.3	32.8

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1975

Per Cent Burn															
Years	0-30			30-40			40-50			50-60		60-100			
	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%
	Pts. Deaths Mortality			Pts. Deaths Mortality			Pts. Deaths Mortality			Pts. Deaths Mortality			Pts. Deaths Mortality		
1962-63	140	6	4.3	35	16	44.4	36	22	61.1	23	18	78.3	55	49	89.1
1964-75	1600	50	3.0	505	91	18.0	423	134	31.7	275	137	49.8	494	421	85.2

from the Continental United States. Table 2 summarizes the burn etiology in 1975. Table 3 summarizes the effect of age and total body surface burn on mortality.

Table 4 lists the mortality rates in increments of 10% total body surface from the year 1971 through 1975. Table 5 presents the survival and mortality rate of patients with greater than 30% body surface burns in the calendar years 1955-1975. It should be noted that there are no marked changes. Table 6 shows a comparison of burn mortality in the pre-topical antimicrobial years 1962-1963 with the cumulative index since 1965 when Sulfamylon and later silver sulfadiazine have been used. As previously reported the improvement is primarily in that group of burn patients whose injury is 30-60% with little or no change in those patients with greater than 60% burn injury.

The average hospital stay in 1975 was 49.8 days. When convalescent leave for active duty military personnel was excluded the average hospital stay was 42 days. The average post burn day of admission to the Institute of Surgical Research was 2.9. This figure reflects the decrease in the average post burn day of admission from 11.2 days in 1970 to 9 days in 1971 and 7 days in 1972 and 5 days in 1973, and was the same as the 2.8 days in 1974. The decrease in the average admission time is because the patient population originates in the Continental United States and also reflects the rapid aeromedical evacuation currently practiced at the Institute of Surgical Research.

During the year 1975 1,201 operations were performed on 211 patients, or an average of approximately 5 operations per patient. Four hundred and ninety anesthetics were performed on 142 patients or three anesthetics per patient participating. The total of 670 ward procedures were performed. Three hundred and thirty-one autografting procedures were carried out on 114 patients or approximately 2 1/2 procedures per patient. Thirty-one patients had 76 applications of allograft for an average of approximately 2.2 applications per patient participating.

Porcine cutaneous xenograft was applied to 17 patients 28 times or an average of 1 1/2 times per patient. There was a marked decrease in the use of porcine cutaneous xenograft during 1975. Evaluation of this material on wounds ready to accept autograft and wounds not clean enough to autograft revealed that it is clearly inferior to cutaneous allograft. Cadaver allograft was aseptically harvested from 60 cadavers, an increase from the 33 in 1974. Escharotomies were performed on 80 patients or 31.5% of all dispositions. Twenty-five patients required major amputations usually for treatment of severe electric injury. Tracheostomy was performed in 16 patients or 6.3% of all dispositions. This is marked diminution from the 16.4% in 1974. This probably reflects both the decrease in number of early pulmonary complications and a tendency to leave endotracheal tubes in place for longer periods.

One hundred and ninety-two patients or 75.6% of all dispositions

had at least one blood culture drawn during their hospitalization and 115 patients had one or more positive blood cultures. For further information of the bacteriologic data the reader is referred to the succeeding chapters. Suppurative thrombophlebitis occurred in 8 patients or 3% of all dispositions. In keeping with the high index of suspicion of suppurative thrombophlebitis, 35 "cutdown" sites were explored. An important aspect of burn therapy is monitoring the flora of the burn wound and this was done with frequent wound biopsies. Eighty-four patients or 33% of all dispositions in 1975 had at least one burn wound biopsy performed.

A total of 740,885 cc of blood were administered to 164 patients or 64.6% of all dispositions. An average of 4,518 cc of blood were given to each of the 164 patients. Thirteen patients or 5.1% of all dispositions had a major fracture upon admission. Two patients were also admitted with hip dislocations and two with traumatically open knee joints. The aforementioned 25 major amputations were all related to electrical injuries with the exception of two patients.

There were frequent ophthalmologic injuries in the 254 dispositions in 1975 which included corneal burns, corneal abrasions, corneal lacerations but mainly thermal injuries to the lids. Significant long term sequelae of eyelid burns occurred in the form of all degrees of ectropion. Of the surviving patients in 1975 there was only one with permanent visual impairment, that occurring secondary to a corneal scar from a deep corneal burn. Again in 1975 chondritis in the thermally injured ear has been a major problem. In 1975 27 patients or 10.6% of all dispositions had chondritis. This is double the incidence in 1974 and continues to underline our failure in understanding this process. The current treatment has emphasized keeping pressure off of burned ears and keeping them covered with a topical antimicrobial agent, however a good portion of the chondritis seems to be unresponsive to these procedures.

Gastrointestinal complications played a lesser role in 1975. Although some GI bleeding was noted in 60 patients, the bulk of these had only guaiac positive stools and frank ulceration was diagnosed in only 33 patients or 12.9% of all dispositions, the majority of these were diagnosed at post mortem examination and many were considered to be terminal changes. There was only one GI bleed severe enough to require surgery during 1975 but there were three ulcer perforations, all occurring as terminal events. This lack of significant GI complications may well reflect the effectiveness of prophylactic antacid neutralization of gastric acid in all early post burn patients. Superior mesenteric artery syndrome was diagnosed radiographically in three patients; all were managed conservatively and had favorable outcomes. This stands in contrast to 1974 when three were diagnosed, and two were operated upon with unfavorable outcomes. It would seem from this small series that a vigorous trial at conservative therapy is indicated prior to operative intervention. The only other significant GI complication occurred in a patient with idiopathic atony of the right colon which eventually reached 16 centimeters in diameter and required a cecostomy for decompression.

Major renal complications in our burn population occurred in 43 patients or 17% of all dispositions. This was usually a terminal event often related to sepsis expressed as some degree of renal failure. In fact the bulk of the renal problems in the burn patients appear to be secondary to sepsis. Hemodialysis was carried out in nine patients with no survivors.

Cardiac complications continued to play a significant role in burn patient morbidity and mortality. Again as in 1974 nine patients sustained acute myocardial infarction and again all succumbed from the insult. As in 1974 there have been several episodes of acute bacterial endocarditis with staph aureus coagulase positive being the causative organism. Four cases of endocarditis were diagnosed in 1975 as compared to five in 1974 however there were multiple patients that had blood cultures positive for Staphylococci which cleared on therapy in whom the source of bacteria remained undefined. This lesser incidence of staphylococcal endocarditis may reflect the increased awareness of the lethal potential of Staphylococcal septicemia in the burn patient and the beneficial effect of starting antibiotic therapy with the first positive culture.

Pulmonary problems continued to be a significant cause of morbidity and mortality in 1975 as they have in the past. However, early pulmonary problems appear to have decreased in frequency except for those of inhalation injury. There were 60 patients with inhalation injuries documented on ¹³³Xenon lung scan and bronchoscopy or an incidence of 23.6% of all dispositions. It is again apparent that inhalation injury significantly increases the mortality in patients with 30 to 65% burns and has little if any effect on the mortality of patients with burns of more than 65%.

SUMMARY

During 1975 245 patients were admitted to the U.S. Army Institute of Surgical Research and 254 dispositions were made. Silver sulfadiazine was continued as the initial burn therapy however, it appears to be sorting out a more resistant Klebsiella as documented by earlier time of positive blood culture, earlier onset of sepsis and earlier time to death. Again there were fewer significant pulmonary complications and the patients had a much more benign early post burn course. Infection continues to be the most common cause of mortality in the thermally injured patient. Clinical research efforts are oriented towards evaluation and control of the septic process with ongoing research on the efficacy of various topical agents, the role of hydrotherapy in the management of burn wounds, the use of systemic antibiotics, immunosuppression with excision and allografting in massive burns and other means to improve the survival of the burned soldier.

Table 7. Causes of Death, 1975

Patient	Age	Sex	% Burn Total	3 rd	PBD Death	Cause of Death
1	9	M	95	91	21	Septicemia (staphylococcal and Pseudomonas), severe "adult hyaline membrane disease"
2	36	M	95	62.5	1	Severe inhalation injury
3	50	M	92.5	80.5	7	Severe inhalation injury
4	18	M	90.5	55	10	Gram-negative sepsis (Enterobacter, Pseudomonas)
5	15	F	90	80	7	Sepsis (Klebsiella); acute renal failure
6	11	M	89	79	96	Severe hyaline membrane disease, severe bronchopneumonia
7	68	F	85	60	1	*Cardiovascular collapse
8	63	M	85	54	1	Inhalation injury and cardiovascular collapse
9	28	M	84.5	72	4	Severe inhalation injury
10	70	M	83	41	3	Severe inhalation injury
11	26	M	82.5	47	15	Sepsis (Enterobacter, Klebsiella and Pseudomonas)
12	22	M	82	49	5	Severe inhalation injury
13	25	F	82	43	14	Septicemia (Klebsiella, Enterobacter), gastric ulcer with hemorrhage
14	53	M	80.5	57.5	12	*Severe inhalation injury
15	27	F	80.5	18	10	*Septicemia (Klebsiella)
16	27	M	80	50	6	*Severe inhalation injury; septicemia (Pseudomonas)
17	40	M	80	22	15	Severe bronchopneumonia with abscess formation organism Staphylococcus aureus coagulase positive

*Autopsy not performed

Table 7. Causes of Death, 1975

Patient	Age	Sex	% Burn Total	PBD Death	Cause of Death	
18	53	F	75.5	7	4	Aspiration of gastric contents
19	46	M	79	16	41	Burn wound sepsis organisms Klebsiella, liver failure
20	65	M	78.5	67	4	Arteriosclerotic heart disease manifested by acute myocardial infarction
21	41	M	78	15	19	Arteriosclerotic heart disease manifested by acute myocardial infarction
22	18	M	78	49	65	Acute bacterial endocarditis organism Staphylococcus aureus coagulase positive
23	28	F	77.5	34.5	15	Severe pneumonia secondary to inhalation injury
24	34	M	76.5	19	8	*Severe inhalation injury
25	45	M	76	58	2	Severe inhalation injury
26	28	M	75.5	43	3	Multiple pulmonary emboli
27	85	F	75.5	29	2	Cardiovascular collapse
28	29	M	73	41	13	Inhalation injury, sepsis (Enterobacter and Klebsiella)
29	3	F	73	38.5	37	Sepsis organism Pseudomonas
30	21	M	73	33.5	9	Cardiovascular collapse
31	58	M	71	54	12	Sepsis organism Klebsiella
32	23	F	71	0	2	*Severe inhalation injury
33	42	M	70.5	1.5	17	Burn wound sepsis, organism Pseudomonas
34	28	M	69.5	56	44	Acute bacterial endocarditis organism Staphylococcus
35	25	F	69.5	53.5	11	Multiple pulmonary emboli

*Autopsy not performed

Table 7. Causes of Death, 1975

Patient	Age	Sex	% Burn Total	POB 3 rd Death	Cause of Death
36	65	M	69.5	32.5	9 Severe lobar pneumonia
37	17	M	69	36.5	36 *Sepsis organism Staphylococcus
38	49	M	68	33.5	13 *Staphylococcal septicemia and severe inhalation injury
39	26	M	68	30	31 Pseudomonas burn wound invasion
40	23	F	68	22	23 Mycotic burn wound invasion, mycotic abscess left temporal lobe, severe pneumonia
41	18	F	67	43	25 *Burn wound invasion organism Pseudomonas
42	24	M	67	39.5	23 Severe inhalation injury, sepsis Enterobacter and Klebsiella
43	54	M	67	35	3 Severe inhalation injury
44	26	M	66	40	9 Diffuse bronchopneumonia and cardiomyopathy, secondary to Friedreich's ataxia
45	6	F	66	10	18 Aspiration gastric contents
46	19	M	66	5.5	6 Severe inhalation injury and bronchopneumonia organism Klebsiella
47	20	M	65.5	46	17 Sepsis organism Klebsiella
48	30	M	65	42	9 Severe pneumonia organism Pseudomonas
49	21	M	65	40.5	15 Sepsis (Klebsiella), aspiration gastric contents
50	2	M	64	59	14 *Septicemia organism Klebsiella
51	20	F	63	14	15 Inhalation injury
52	28	F	62	0	15 Septicemia, Staphylococcal
53	18	F	61.5	56	38 Staphylococcal sepsis secondary to acute bacterial endocarditis

* Autopsies not performed

Table 7. Causes of Death, 1975

Patient	Age	Sex	% Burn Total	3°	P80 Death	Cause of Death
54	40	M	61	21	5	*Sepsis organism E. coli
55	67	M	60	3	6	*Acute renal failure and acute cerebral edema
56	61	M	60	0	9	Sepsis organism, Klebsiella
57	23	F	57.5	13	16	Inhalation injury and acute necrotizing bronchopneumonia
58	42	M	57.5	3.5	10	*Sepsis organism Klebsiella
59	28	M	57	44	1	Multiple trauma including multiple fractures, laceration right iliofemoral vein, massive intra-abdominal hemorrhage
60	32	M	55	30.5	14	*Inhalation injury, bronchopneumonia
61	33	M	54.5	43	28	Sepsis, organism Klebsiella
62	21	M	54.5	11	8	Inhalation injury and severe bilateral bronchopneumonia
63	27	M	53	4	8	Burn wound invasion organism Klebsiella and Pseudomonas
64	47	M	53.5	26	7	*Severe inhalation injury and bronchopneumonia
65	47	M	51	31	7	Sepsis organism Pseudomonas
66	6/52	F	51	31	10	Burn wound invasion multiple organisms, hematogenous pneumonia
67	46	F	50.5	35.5	23	Sepsis and inhalation injury
68	27	M	50	31.5	1	Severe inhalation injury
69	47	M	50	50	1	*Severe inhalation injury
70	54	M	50	44	12	Sepsis organism Enterobacter
71	58	M	49.5	42.5	10	Arteriosclerotic heart disease manifested by acute myocardial infarct

* Autopsy not performed

Table 7. Causes of Death, 1975

Patient	Age	Sex	% Burn Total	PBD 3° Death	Cause of Death	
72	45	M	49	6	34	Burn wound sepsis organism <i>Staphylococcus</i> and <i>Pseudomonas</i>
73	1	M	48	22.5	8	Septicemia organism <i>Klebsiella</i>
74	18	M	47	26	29	*Septicemia organism <i>Staphylococcus</i>
75	33	M	46.5	3	42	Inhalation injury, severe hyaline membrane disease secondary to inhalation injury and respirator
76	52	M	42	32	20	*Septicemia organism <i>Enterobacter</i> and <i>Staphylococcus aureus</i> coagulase positive
77	68	M	42	12.5	4	Cardiovascular collapse secondary to severe arteriosclerotic heart disease
78	72	M	41.5	30	14	Diffuse bronchopneumonia and recent cerebro-vascular accident
79	48	M	39	31	1	Inhalation injury and cardiovascular collapse
80	45	M	36.5	34.5	17	Sepsis organism <i>Klebsiella</i>
81	43	M	36	2	33	Gastrointestinal hemorrhage secondary to cecal ulcer and constrictive pericarditis
82	61	M	35	19	29	Severe arteriosclerotic heart disease and aspiration pneumonia
83	24	M	34.5	6.5	11	Inhalation injury severe, bilateral pneumonia, acute bacterial endocarditis organism <i>Staphylococcus aureus</i> coagulase positive
84	28	M	34	25.5	4	*Disseminated intravascular coagulopathy and respiratory failure
85	40	F	33	19	13	Sepsis organism <i>Klebsiella</i>
86	50	W	33.5	0	10	Inhalation injury with severe bilateral bronchopneumonia secondary to inhalation injury

*Autopsy not performed

Table 7. Causes of Death, 1975

Patient	Age	Sex	% Burn Total	3°	PBD Death	Cause of Death
87	13	M	32	26	27	*Staphylococcal septicemia
88	66	M	30	30	24	*Inhalation injury and septicemia organisms Klebsiella and Pseudomonas
89	21	M	30	30	123	Severe cardiomyopathy etiology unknown
90	57	M	28	0	5	Severe inhalation injury
91	50	M	25	20	21	Cardiovascular insufficiency secondary to severe arteriosclerotic heart disease
92	54	M	20	17	15	Sepsis, organism Klebsiella and renal failure
93	64	F	20	10.5	9	*Inhalation injury with resultant respiratory failure
94	77	F	18.5	3.5	34	Multiple pulmonary emboli

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Berry DM: Nursing Research in the U.S. Army. ANC Officers, BAMC Fort Sam Houston, TX 20 Jan 75.

Peterson HD: Recent Advances in Burn Therapy. San Antonio Society of Insurance Underwriters, San Antonio, TX 20 Jan 75.

The following presentations were made to the Physical Therapy students Academy of Health Sciences, Fort Sam Houston, TX 27 Jan 75:

Michael MG: Nursing Care of Burned Patients

Long JM III: Current Therapy of Burns

Fuller P: Introduction to Current Concepts of Burn Nursing

Jesse NF: Physical Therapy for the Burn Patient

Shaw AL: Occupational Therapy for the Burn Patient

Long JM III: Principles of Intravenous Hyperalimentation. Eastern Virginia Society of Hospital Pharmacists and Norfolk area nurses. Norfolk, VA 29 Jan 75.

Long JM III: Comparison of Carbohydrate and Fat as Caloric Sources for Total Intravenous Feeding. South Texas Chapter of the American College of Surgeons mtg, Galveston, TX 31 Jan 75.

Agee RN. Classification of Burns. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 3 Feb 75.

Taylor JW: Burn Wound Therapy. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 5 Feb 75.

Erickson DR: Complications of Burns. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 6 Feb 75.

The following presentations were given at the Inservice Program for the Brooke Army Medical Center Nursing Service on 5 and 13 Feb 75:

Berry DM: Nursing Research

Michael MG: Current Concepts of Burn Nursing
Podgornoff WC: Intermediate and Convalescent Care
Zuber D: Care During the Acute State of Injury

Long JM III: Hyperalimentation. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 12 Feb 75.

Fuller P: Current Concepts of Burn Nursing. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 13 Feb 75.

Wilmore DW: Applied Science in the Care of Critically Ill Patients. Engineer Conference for Texas High School Seniors, Univ of Texas, Austin, TX 14 Feb 75.

Long JM III: Burn Injury: A Challenge to Patient, Doctor and Community. North Austin Rotary Club. Austin, TX 17 Feb 75.

Wilmore DW: Use of Intravenous Fat Emulsions. San Antonio Surgical Society, San Antonio, TX 18 Feb 75.

Long JM III: 1) Clinical Aspects of Intravenous Hyperalimentation. Loma Linda area Pharmacists, Nurses and Physicians. 2) What's New in Burn Therapy. Surgical Grand Rounds. Loma Linda University. 3) Problems in the Care of Burned Patients. Nurses of the Intensive Care Unit and Burn Unit. Loma Linda, CA 19 Feb 75.

Long JM III: Clinical Aspects of Intravenous Hyperalimentation. Inservice seminar. Santa Rosa Medical Center, San Antonio, TX 21 Feb 75.

Taylor JW: Treatment of Burns
Podgornoff WC: Nursing Care of Burns. Nursing students San Antonio College, San Antonio, TX 25 Feb 75.

Wilmore DW: Uses and Abuses of Parenteral Nutrition. Columbia Univ, NY 7 Mar 75.

The following presentations were given to the Overseas Industry Research Center Study Group on 10 Mar 57:

Pruitt BA Jr and Welch GW: Early Care of the Burn Patient, Including Fluid Resuscitation

Peterson HD: Wound Care - Topical Therapy

Levine NS: Wound Care - Excision

Taylor JW: Wound Care - Wound Closure

Long JM III: Metabolic Consequences of Thermal Injury and Nutrition of the Burn Patient

Petroff PA and Hander EW: Pulmonary Changes Following Thermal Injury

Czaja AJ: Gastrointestinal Complications of Thermal Injury

Merrill RH and Myers WD: Renal Changes Following Thermal Injury

Andes WA: Hematologic Changes Following Thermal Injury

Rizzo TA Jr: The Burn Autopsy and Burn Wound Biopsies

Michael MG: Orientation to the Nursing Care of the Burned Patient. 156th Aeromedical Evac Flt, NC Air National Guard, Charlotte, NC 12 Mar 75.

Long JM III: Clinical Aspects of Intravenous Hyperalimentation. South Illinois Soc of Hosp Pharmacists and Champaign-Urbana area nurses. Champaign, IL 14 Mar 75.

Wilmore DW: Metabolism Following Injury. Univ of Michigan Sch of Med, Ann Arbor, MI 14 Mar 75.

Taylor JW: Burns. Boy Scouts of America. San Antonio, TX 17 Mar 75.

The following presentations were made at the American Burn Assn Anl mtg in Denver CO 20-22 Mar 75:

Levine NS: Limitations in Applying Tangential Excision to Large Burns

Taylor JW: Scalp as a Donor Site

Czaja AJ: Acute Liver Disease and Cholestatic Jaundice After Cutaneous Thermal Injury.

McAlhany JC: Perforated Curling's Ulcer: Incidence, Diagnosis and Therapy

Agee RN: Xenon¹³³ Lung Scan for Early Diagnosis of Inhalation Injury

Wilmore DW: Attempts to Modify the CNS Directed Stress Response to Thermal Injury

Petroff PA: Ventilatory Patterns Following Burn Injury and Effect of Sulfamylon

Long JM III: Effect of Carbohydrate and Fat Intake on Nitrogen Excretion During Total Intravenous Feeding

Michael MG: Inservice in a Folder

Baskin TW: Acute Bacterial Endocarditis A Silent Source of Sepsis in the Burn Patient

Wilmore DW: Use of Nutrition and Environmental Control for Burn Patients.

Wilmore DW: Recent Care of the Thermally Injured. R.N. Club, Fort Sam Houston, TX 18 Mar 75.

Long JM III: Problems and Potentials of Intravenous Therapy. Illinois State Soc of Hospital Pharmacists, Chicago, IL 27 Mar 75.

Long JM III: 1) Clinical Use of Total Intravenous Feeding and, 2) Team Approach to IV Hyperalimentation. Physicians, Nurses, and Pharmacists. Clearwater, FL 1 Apr 75

Wilmore DW: Metabolism and Nutrition in the Septic Patient. Wayne State University, Detroit, MI 5 Apr 75.

Long JM III: Intravenous Hyperalimentation. Louisiana Soc of Hosp Pharmacists and nurses. New Orleans LA 4 Apr 75.

Peterson HD: Burn Complications. Nursing Inservice Program. BAMC Fort Sam Houston, TX 29 Apr 75.

Peterson HD: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 30 Apr 75.

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 2 May 75.

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 5 May 75.

Long JM III: Intravenous Hyperalimentation. Gastroenterology Service, BAMC, Fort Sam Houston, TX 12 May 75.

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 16 May 75.

Long JM III: Intravenous Hyperalimentation. Central Texas Society of Hospital Pharmacists, San Antonio, TX 20 May 75.

Wilmore DW: 1) The Role of the Central Nervous System Following Injury; 2) Catecholamines: Mediator of the Hypermetabolic Response Following Injury; 3) Nutritional Support in Injured Patients. International School of Medical Science, Erice, Italy 19-28 May 75.

Long JM III: What's New in Burn Therapy. Staff of Harrisburg Polyclinic Hospital, Harrisburg, PA 4 Jun 75.

Long JM III: Use of IV Fat Emulsion in Burns and Trauma. AMA Symposium on Intravenous Fat Emulsions. Chicago, IL 6 Jun 75.

Wilmore DW: Essential Fatty Acid Deficiency in Burn Patients. AMA Symposium of Fat Emulsions. Chicago, IL 6 Jun 75.

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 10 Jun 75.

Long JM III: Practical Aspects of Total Intravenous Feeding. IV Therapy Seminar at the University of Indiana Medical Center, Indianapolis, IN 11 Jun 75.

Peterson HD: Current Therapy in Burns. Regional Meeting of San Antonio Society of Insurance Underwriters. San Antonio, TX 13 Jun 75.

Wilmore DW: Metabolism Following Thermal Injury. Univ of Virginia, Richmond, VA 13 Jun 75.

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 17 Jun 75.

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 24 Jun 75.

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 1 Jul 75.

Rosenthal A: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 21 Jul 75.

Rosenthal A: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 4 Aug 75.

Peterson HD: Plastic Surgery in the Military. Senior Army Chaplains BAMC CPE Chaplains Course, Fort Sam Houston, TX 7 Aug 75.

Slade CL: Classification of Burns - Initial Care. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 12 Aug 75.

Slade CL: Burn Wound Therapy. Students Intensive Care Nursing Course, BAMC, Fort Sam Houston, TX 14 Aug 75.

Rosenthal A: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 18 Aug 75.

Erickson D: Complications of Burns. Students Intensive Care Nursing Course, BAMC, Fort Sam Houston, TX 19 Aug 75.

Slade CL: Hyperalimentation Therapy. Students Intensive Care Nursing Course, BAMC, Fort Sam Houston, TX 21 Aug 75.

Hayward C: Nursing Care of the Burn Patient. Students Intensive Care Nursing Course, BAMC, Fort Sam Houston, TX 26 Aug 75.

Peterson HD: Burns in the Multiple Trauma Patient. Sixth International Congress of Plastic and Reconstructive Surgery, Paris France. 26 Aug 75.

Pruitt BA Jr: Early Care of the Extensively Burned Patient. General Surgery staff, residents, interns, Wilford Hall Med Ctr, Lackland AFB, TX 29 Aug 75.

Pruitt BA Jr: Gram-Negative Infections in the Burn Patient. Symposium on Gram-Negative Infections, Baylor College of Medicine, Houston, TX 30-31 Aug 75.

Rosenthal A: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 4 Sep 75.

Pruitt BA Jr: The Early Treatment and Complications of Burn Injury in Aged and Other High Risk Patients. Stanford Univ Sch of Med, Stanford CA 4 Sep 75.

Peterson HD: Burn Assessment and Management. Interns Brooke Army Medical Center, Fort Sam Houston, TX 8 Sep 75

Wilmore DW: The Neurohumoral Control of the Post-traumatic Changes. European Injury Meeting, Glasgow, Scotland 12 Sep 75.

Rosenthal A: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 22 Sep 75.

The following presentations were made to the student nurses of San Antonio College on 23 Sep 75:

Rosenthal A: The Treatment of Burns

Taylor D: Nursing Care of the Burn Patient

Pruitt BA Jr: Discussor for three papers: 1) Sepsis--Prevention and Management; 2) Respiratory and Gastrointestinal Complications; 3) The Future for Burn Care. American Burn Assn mtg, Kansas City, MO 25-27 Sep 75.

The following presentations were made and meetings attended by Pruitt BA Jr in connection with the ACS mtg San Francisco, CA 12-17 Oct 75: 1) Mtg, Editorial Subcommittee for Manual on Control of Infections; 2) Mtg, ACS Board of Governors; 3) Mtg ACS Executive Committee, Pre-and Postop Care; 4) Mtg Surgical Biology Club III; 5) Mtg ACS Committee on Trauma (Publications); 6) ABA Board of Trustees mtg; 7) ACS Pre and Postop Care Committee Mtg; 8) Mtg US Chap Internatl Soc of Surgery; 9) Panel Postgraduate Course No. 13 "Management of Facial Injury" presentation: The Acutely Burned Face; 10) ABA Ad Hoc Comm on Standards; 11) Adjourned mtg, ACS Board of Governors.

Wilmore DW: Alterations in Glucose Kinetics Following Thermal Injury. ACS Mtg, San Francisco, CA 15 Oct 75.

Rosenthal A: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 16 Oct 75.

Peterson HD: Prospective Comparison Sulfamylon and Silver Sulfadiazine. American Soc of Plastic and Reconstructive Surgeons, Inc., Toronto, Canada 21 Oct 75.

Wilmore DW: Parenteral Nutrition in Burn Patients. Massachusetts General Hospital, Boston, MA 29 Oct 75

Peterson HD: Plastic Surgery in the Military. Plastic Surgery Service, Wilford Hall USAF Hospital, Lackland AFB TX 30 Oct 75.

Pruitt BA Jr: Burns. Department of Surgery, Fort Leonard Wood Army Hospital, MO 6 Nov 75.

Peterson HD: Plastic Surgery. Third Annual Institute of the Assn of Operating Room Technicians of San Antonio, TX 8 Nov 75.

Pruitt BA Jr: Burns. Panel Member, Emergency Medical Service Patient Care Systems Design and Implementation Symposium, Grand Rapids, MI 11-13 Nov 75.

Pruitt BA Jr: 1) Prevention and Treatment of Sepsis in Burn Patients
2) Respiratory and Gastrointestinal Complications of Burn Patients; and
3) Unsolved Problems in Burn Care. ABA Seminar, Birmingham, AL 14-16 Nov 75.

Rosenthal A: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 17 Nov 75.

Peterson HD: Modern Burn Therapy. Medical Aspects of Advanced Warfare Course, Brooks AFB, TX 19 Nov 75.

Peterson HD: Recent Advances in Burn Management. General Surgery Service, University of Mississippi and Treatment of the Burned Hand, Plastic Surgery Service, Univ of Mississippi, Jackson, MS 21 Nov 75.

Pruitt BA Jr: Importance of Burn Units, Role in Education and Prevention and Patient Care Dedication of Baptist Medical Center Burn Center, Oklahoma City, OK 22 Nov 75.

Wilmore DW: Energy and Protein Metabolism in Disease. Ross Conference, Amiele Island, FL 22 Nov 75.

Pruitt BA Jr: Opportunistic Infection in Burn Patients. Charlotte Surgical Society, Visiting Professor Charlotte Memorial Hospital, Charlotte, NC, 24-26 Nov 75.

Pruitt BA Jr: Burn Wound Care. Surgical Staff, Wilford Hall Medical Center, Lackland AFB, TX 5 Dec 75.

The following presentations were made at the mtg of the Massachusetts Chapter of the APTA Workshop, Framingham, MA 4-6 Dec 75:

Peterson HD: 1) Early Medical Management of Burn Patients 2) Nutritional Management. Prevention and Treatment of Infection in the Burn Patient. 3) Skin Grafts and Wound Coverage for Burn Patients.

4) Reconstructive Surgery and Management for Burn Patients. 5) Inhalation Injuries of Burn Patients.

Roberts ML: 1) Nursing Care in the Acute Phase. 2) Continued Nursing Care

Jesse F and Shaw A: Acute Phase of Burn Treatment. 2) Long Term Care Phase of Burn Patients.

Rosenthal A: Burns - Assessment and Management, Interns BAMC Fort Sam Houston, TX 8 Dec 75.

Rosenthal A and Taylor D: Management of Burns. Physical Therapy students, Academy of Health Sciences, Fort Sam Houston, TX 9 Dec 75.

Pruitt BA Jr: Pulmonary Complications of Thermal Injury. Plastic Surgical Symp of Nassau Surgical Soc, New York, 10 Dec 75.

Pruitt BA Jr: 1) Resuscitation and Early Care of the Severely Burned Patient; 2) Home Remedies and the History of Burn Care; 3) Wound Care and Opportunistic Infections in Burn Patients. Visiting Professor at State Univ of New York at Stony Brook, Stony Brook, NY 11-12 Dec 75.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
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10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3A161102B71R		01		168	
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Clinical Operation, Surgical Study Branch, For Treatment of Injured Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
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17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
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20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: Douglas W. Wilmore, M.D.			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-5712			
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FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: L. Howard Aulick, Ph.D., MAJ, MSC			
				NAME: Basil A. Pruitt, Jr., COL, MC DA			
22. KEYWORDS (Precede with Security Classification Code) ^a (U) Trauma; (U) Combat casualties; (U) Laboratory animals; (U) Thermally injured soldiers; (U) Wound healing; (U) Gastrointestinal pathology							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
23. (U) Clinical and laboratory investigations pertaining to severe physical trauma which has been sustained by soldiers in the field.							
24. (U) Planned clinical and laboratory studies relating to acute and chronic effects of injury. Studies conducted in this branch have included both clinical investigation involving patients and normal individuals and laboratory studies involving animal models.							
25. (U) 75 07 - 76 09 The Surgical Study Branch continued investigations of the metabolic response to thermal injury and the effects of physical trauma on wound healing, susceptibility to infection, and gastrointestinal and liver function. The effects of nutritional support on posttraumatic metabolic response and organ system alteration is also being evaluated. The metabolic kitchen provides support for these investigations, in addition to preparation of specialized diets for patients with feeding problems, and the provision of diets containing known constituents to be used in a wide variety of metabolic balance studies. In addition, members of the branch provide nutritional, gastrointestinal and physiological consultation for all institute patients and other individuals outside the institute with complex gastrointestinal, metabolic, nutritional and temperature regulation problems.							

^aAvailable to contractors upon originator's approval

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1 MAR 68

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TERMINATION REPORT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: CLINICAL OPERATION, SURGICAL STUDY BRANCH FOR
TREATMENT OF INJURED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

Douglas W. Wilmore, MD
Louis H. Aulick, PhD, Major, MSC
John P. Peterson, Captain, AMSC
David H. Dahlquist, SP5, EM

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

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TREATMENT OF INJURED SOLDIERS.

US Army Institute of Surgical Research, Brooke Army Medical Center,
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Reports Control Symbol MEDDH-288(R1)

This branch studies the physiologic alterations which occur following severe injury and interrelates the changes in circulation, metabolism, temperature regulation, and hormonal control with alterations in the wound and the whole patient. The design and construction of a whole leg plethysmograph for measurement of leg blood flow and development of an on-line technique for continuous oxygen consumption measurement have been major technical advances in support of this investigative effort. Branch members provide consultative services to departments of the Brooke Army Medical Center and the University of Texas Health Science Center at San Antonio. Branch members are actively engaged in teaching programs on a local, national, and international level.

Surgical study
Surgical physiology

CLINICAL OPERATION, SURGICAL STUDY BRANCH FOR TREATMENT OF INJURED SOLDIERS

The three major activities of the Surgical Study Branch are: 1) delivery of medical and surgical care to the thermally injured soldier admitted to this institute; 2) clinical and laboratory research in problems related to care and rehabilitation of burn patients, and application of this knowledge to other critically ill patients; 3) the education of medical and paramedical personnel in the care of the seriously injured.

Members of this branch round daily to evaluate the patient problems in the Clinical Division of the US Army Institute of Surgical Research. The branch chief serves as coordinator of all clinical research, and all branch members provide consultative service and care in the areas of post-traumatic metabolism and nutrition, energy balance, gastrointestinal and hepatic dysfunction, peripheral circulation, and temperature regulation. Branch members provide consultative services for General Surgical, Medical, Endocrinology, Obstetrics and Gynecology, Gastroenterology, Pediatrics, and Dermatology Services at the Brooke Army Medical Center and the University of Texas Health Sciences Center at San Antonio. Techniques and modalities developed in this unit are currently applied to the care delivered to seriously injured patients who remain hospitalized until all wounds are healed.

Clinical and laboratory research in this division may be placed in the following categories: 1) temperature regulation; 2) post-traumatic metabolism; 3) energy balance; 4) nutritional support of critically ill soldiers; 5) relationship between peripheral circulation and substrate flow to the burn wound; 6) description of hepatic dysfunction which occurs following injury, with specific emphasis on the interrelation between hepatic glucose production, splanchnic blood flow, and energy production of the burn patient. Two technical developments have aided in these areas: 1) construction of a whole leg plethysmograph, and 2) development of an on-line continuous measurement system for total body oxygen consumption (by Mr. Edwin W. Hander). In addition, the development of a hypermetabolic animal model by Dr. David Herndon has greatly broadened the scope of physiological and nutritional studies which can be accomplished.

Branch members participate actively in teaching activities of this unit, the Brooke Army Medical Center, and are affiliated on the staff of the Medical School of the University of Texas at San Antonio. In addition, Branch members have actively participated in local, national, and international meetings to present and discuss their research findings and increase the scientific interchange in these areas of study.

PUBLICATIONS AND/OR PRESENTATIONS:

See report of Clinical Division, USAISR

TERMINATION REPORT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: ANESTHESIOLOGY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1975 - 31 December 1975

Investigators:

Gary W. Welch, MD, Ph.D, Lieutenant Colonel, MC
James K. Sims, MD, Major, MC
Allister K. Morris, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: ANESTHESIOLOGY

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 January 1975 - 31 December 1975

Investigators: Gary W. Welch, MD, Ph.D., Lieutenant Colonel, MC
James K. Sims, MD, Major, MC
Allister K. Morris, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

In the period covered in this report, 490 anesthetics were administered to 142 patients, an average of 3.45 anesthetics per patient. Although most patients received only two or three anesthetics, several received over 10 anesthetics.

The most commonly used anesthetic was ketamine followed by nitrous oxide and ethrane. Regional anesthetics were administered to only a small per cent.

Three cases of difficult anesthetic management are presented.

Anesthesia

ANESTHESIOLOGY

PREOPERATIVE EVALUATION

With the exception of patients with electrical injury, most burn patients scheduled for surgery are several days to weeks postinjury. In the immediate postburn period, patients have the determination of electrolytes, arterial blood gases, hematocrit, and other hematologic parameters determined as indicated. Blood pressure, central venous pressure and urine output are recorded on an hourly basis. During the past year, adult patients with a thermal injury greater than 40% have had the Swan-Ganz thermal dilution flow directed pulmonary artery catheter inserted, allowing frequent determination of the cardiac index. Daily chest x-rays are also obtained. Hence, all patients coming to the operating room have abundant physiologic data available upon which anesthetic decisions may be made. In addition, a preoperative electrocardiogram is obtained in all electrical injuries to determine the possible presence of myocardial injury.

PREOPERATIVE PREPARATION

All adult patients are kept NPO past midnight. Exceptions to this are patients with a tracheostomy or nasotracheal tube in place who may be receiving tube feeding. Because the airway is protected in these patients, nutritional support may be continued up to the time of surgery depending upon the age and size of the patient.

PREOPERATIVE ASSESSMENT AND FLUIDS

Premedication varies from none to heavy doses of narcotic. The usual premedication is a combination of either demerol or morphine and glycopyrolate. In the past year, intramuscular administration of diazepam has been replaced by oral administration. Two factors account for this: 1) it is a painful injection; 2) blood levels are nearly the same for both routes of administration. Pediatric cases may be given a small dose of ketamine prior to coming to the operating room. All fluids being given to the patient are changed to D₅RL on arrival in the operating room.

TYPES OF ANESTHESIA

A. General Anesthesia

1. Ketamine

Ketamine, a phencyclidine derivative, produces a dissociative state upon intravenous administration. It has two major advantages. The first is maintenance of ventilation during the anesthetic

state, and the second advantage is the lack of depression of the cardiovascular system. Hence, patients with hypotension or compromised cardiovascular system can be induced relatively safely with ketamine. Although the airway is usually protected, obstruction or regurgitation and aspiration may occur. Ketamine is particularly useful in procedures around the face or for difficult surgical positions. Thirty-two per cent of anesthetics given over the past year were ketamine.

2. Nitrous oxide with barbiturate or narcotic supplementation

Used approximately 28% of the time, this technique allows for rapid emergence by reversal of the narcotic at the end of the procedure. Patients to be intubated while awake or ventilated postoperatively are often anesthetized using a narcotic for sedative effect. Most frequently, this technique is used in conjunction with a nondepolarizing muscle relaxant. In the past year, pancuronium has been used exclusively because of its rapid onset and lack of effect on blood pressure. One possible side effect of pancuronium is a tachycardia following administration of intubating doses (see Complications).

3. Halothane with or without nitrous oxide in oxygen

Although previous research in this unit has shown halothane to be a safe anesthetic in the thermally injured patient, the use of halothane has decreased over the past year. The main advantage of halothane over other inhalational agents is its ability to provide a rapid induction with adequate relaxation of the vocal cords, allowing easy intubation. During the past year, we evaluated serum bromide levels in one patient requiring multiple halothane anesthetics. Serum bromide levels rose after every anesthetic, an indication of increased halothane biotransformation. In the past year, 8% of the anesthetics given were halothane.

4. Enflurane (Ethrane^R)

Enflurane has been available for approximately the past three years. It is a nonflammable fluorinated hydrocarbon which is metabolically stable, less than 2% being biodegraded. Although allowing rapid induction, it is not as smooth an agent as halothane and often relaxation with pancuronium is needed for intubation. To this date, no substantial cases of hepatotoxicity or nephrotoxicity have been reported. In 1975, 27% of the anesthetics given consisted of enflurane.

5. Subanesthetic ketamine

In the past year, the use of low-dose ketamine has been sporadic. For the most part, debridements have been done under general anesthesia in the operating room. The use of subanesthetic ketamine

is not without potential problem because at least two patients have experienced airway difficulty during their procedures in the Hubbard tank.

B. REGIONAL ANESTHESIA

Any patient with a localized lesion who is not septic and is not burned at the site of injection is a candidate for regional anesthesia. That few patients meet such criteria is evidenced by a 2% use of regional for our anesthetics.

MONITORING TECHNIQUES

A. CIRCULATION

1. Precordial and/or esophageal stethoscope.
2. Peripheral monitoring of pulse.
3. Blood pressure by either cuff, Infrasonde^R, or direct intra-arterial line.
4. Central venous pressure.
5. Swan-Ganz catheter.
6. EKG.
7. Sponge weight.
8. Urine output.

B. RESPIRATION

1. Rate.
2. Auscultation.
3. Blood gases as indicated.

C. TEMPERATURE

Prolonged cases and pediatric cases have their temperature monitored during the procedure. Room temperature is maintained at 75-80 degrees Fahrenheit, and the K-thermia heating blanket is reserved either for infants weighing less than 10 kg or to cool febrile patients. Heat lamps are also utilized to warm the patients when needed.

COMPLICATIONS

Case No. 1

Tachycardia following pancuronium administration

This 23 year old patient sustained a 45% thermal injury following a Jeep accident. The areas of injury included the head, buttocks, arms, hands, thighs, and legs. Eight days following injury, he was taken to the operating room for excision of the eschar of his right leg. He was induced with pentothal and given 8 mg pancuronium for intubation. Shortly after intubation, the patient's heart rate was noted to be 200 beats per minute. The operating room monitor did not demonstrate P waves, and, consequently, a Hewlett-Packard EKG machine was brought to the operating room in an attempt to make a diagnosis. It was felt at this time that PAT was the probable diagnosis, and attempts were made to obtain a normal rhythm by carotid massage. This was unsuccessful. Because pancuronium is occasionally associated with tachycardias, it was decided to attempt conversion by reversing the muscle relaxant with edrophonium. Ten milligrams of edrophonium were given with reversion to a normal sinus tachycardia with a rate of 110 beats per minute. Plans for surgery were cancelled, and the patient returned to Ward 14A. During the next several days, the patient continued to have episodes of PAT and eventually he was placed on digoxin, propranolol, and quinidine. This resulted in control of his cardiac rhythm. These medications were continued until discharge, at which time the quinidine and propranolol were discontinued.

Comment

Pancuronium is a nondepolarizing muscle relaxant with a steroidal nucleus. It is rapid in onset, although not as rapid as succinylcholine, with a duration of about 20 minutes. It has been noted to have anticholinergic properties and tachyarrhythmias have been associated with its use. Although the arrhythmia recurred and required propranolol, quinidine, and digoxin for eventual control, pancuronium appears to have been instrumental in initiating PAT in this patient. Subsequent surgical procedures were uneventful.

Case No. 2

Hypotension secondary to bowel necrosis

This patient was a 21 year old male who suffered a 30% thermal injury following an automobile accident. At the time of his arrival in this unit, he had had a left below-knee amputation, the neurovascular bundle in his left axilla was exposed, and he had subcutaneous crepitation. His initial therapy progressed well, but then he developed a myocardopathy and congestive failure.

On the 119th hospital day, the patient suddenly became cyanotic, hypotensive, and acidotic. Abdominal x-rays were consistent with visceral perforation, and the patient was taken to the operating room for exploratory laparotomy. The patient was induced and maintained with ketamine. His blood pressure required continuous support with a dopamine infusion. No urine output was obtained during surgery. At the end of the procedure, the patient was returned to Ward 14A, where he expired shortly thereafter. The operative procedure consisted of right hemicolectomy, terminal ileoectomy, transverse colostomy, and terminal ileostomy. These were done to resect necrotic large bowel.

Comment

Most patients would tolerate a surgical procedure of this magnitude better than this patient did. The patient's underlying myocardiopathy, however, made adequate support of the cardiovascular system nearly impossible.

Case No. 3

Intraoperative myocardial infarction

This patient, a 58 year old male, sustained a 49.5% chemical injury when he fell into a pool of alkaline solution. Three days postinjury, he was scheduled for excision. During his preoperative interview, he admitted to occasional chest pain for which he took no therapy. He claimed he was otherwise healthy and able to carry out his normal activities. For the two days prior to his surgery, he had had cardiac arrhythmias, and he was placed on quinidine and digoxin.

On the day of surgery, the patient was taken to the operating room. He was induced with ketamine and halothane and intubated. For the first hour, the patient's blood pressure remained at 100/50. At that point, it was necessary to remove the blood pressure cuff to debride the arm. Upon reapplication of the cuff, the patient's blood pressure was noted to be 80/40. Throughout the case, the EKG remained stable and CVP varied from 7 to 9 cm of water. Estimated blood loss was 4000 cc, and the patient received 7 units of blood and 2700 cc D₅RL. Upon cessation of the anesthetic, his blood pressure fell to 70 systolic, where it remained on his return to the intensive care unit. A STAT EKG revealed an acute myocardial infarction. Initially, he required pressors for maintenance of his blood pressure, but he was successfully weaned from dopamine. Two days later, it was necessary to return to the operating room for excision of skin from the patient's back and amputation of his left leg. During the procedure, he lost approximately 3500 ml of blood, and he received 6 units of whole blood and one unit of packed cells in addition to 1400 ml D₅RL. Urine output and blood pressure were satisfactory throughout surgery. Postoperatively, the patient remained stable until the 5th postoperative

day, when he developed ventricular instability. Treatment with lidocaine was effective; however, the patient became unresponsive and died that day.

Comment

At autopsy, this patient had severe coronary artery thrombosis and myocardial fibrosis, probably from previous ischemic episodes. Although the preoperative EKG was normal, the patient's history of chest pain would appear to indicate previous ischemic episodes.

The episodes of arrhythmia the patient manifested prior to surgery may have been secondary to myocardial ischemia.

This patient illustrates the risk of anesthesia and surgery in a patient with an acute myocardial infarct. Studies have shown a greater than 50% reinfarction rate, 50% of which are fatal, when a surgical procedure is performed within six months of an infarct. Although aware of this, the nature of the patient's injury required repeated surgical procedures and nothing was available to obviate the risk.

TABLE 1. OVERALL PATIENT DATA, USAISR (1966-1975)

	No. of Patients	No. Patients Anesthetized (ISR Only)	No. Patients Anesthetized No. Patients (x100)	Total Anesthetics (ISR Only)	Anesthetics No. Patients Anesthetized (x100)	Average Per Cent Burn
1966	311	181	58.2	713	3.94	30
1967	389	239	61.4	670	2.80	28
1968	389	259	66.6	794	3.07	30
1969	294	189	64.3	601	3.18	36
1970	321	198	61.7	497	2.51	30
1971	301	179	59.5	475	2.65	31
1972	301	183	60.8	575	3.14	34
1973	273	141	51.6	377	2.67	38.5
1974	226	123	54.4	380	3.09	41.57
1975	254	142	55.9	490	3.45	42.1

TABLE 2. NATURE OF SURGERY, USAISR
(Per Cent)

Procedure	1971	1972	1973	1974	1975
Debridement and/or homograft	15.5	19.7	21.5	22.60	25.0
Autograft	52.9	51.3	52.6	56.9	51.0
Orthopedics	13.0	8.9	8.0	8.1	8.0
Ear (chondrectomy)	4.0	3.1	2.6	1.60	1.0
Eye and lid	3.8	0.7	1.8	1.60	2.0
Intra-abdominal	1.7	7.8	2.1	3.70	2.0
Tracheostomy & bronchoscopy	4.6	6.6	6.6	1.80	<1
Other	4.4	1.9	4.8	3.70	11.0

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OA 6956	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DES'N INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H. TERM	U	U	NA	NL	<input type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
A. PRIMARY	61102A	3A161102B71R		01	141		
B. CONTRIBUTING							
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Clinical Operation, Metabolic Branch, Renal Section, for Treatment of Soldiers With Renal Failure (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
52 07		75 12		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE		EXPIRATION		PREVIOUS		1.0	
B. NUMBER ^a				FISCAL 76		28	
C. TYPE:		D. AMOUNT		YEAR CURRENT		14	
E. KIND OF AWARD		F. CUM. AMT.					
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: William D. Myers, LTC, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-6532			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Richard H. Merrill, LTC, MC			
				NAME			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Hemodialysis; (U) Peritoneal dialysis; (U) Renal failure; (U) Soldiers							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
<p>23. (U) To provide diagnostic support and consultation for the thermally injured soldier and to initiate treatment as is necessary to include both peritoneal and hemodialysis. In addition, the renal section is involved in clinical research activities designed to improve patient care and our understanding of renal problems in the thermally injured soldier. The renal section also supports the clinical and research endeavors of the Nephrology Service at Brooke Army Medical Center in an effort to improve care of military personnel.</p> <p>24. (U) The renal section provides consultation to the physicians involved in direct care of the thermally injured patient. Renal function is assessed by various techniques and therapeutic interventions in the form of hemodialysis and peritoneal dialysis are available. In addition, the renal section is involved in several clinical and laboratory research protocols.</p> <p>25. (U) 75 01 - 75 12 Ten patients were hemodialyzed by the ISR Renal Section for a total of 59 patient treatments. One peritoneal dialysis was carried out. Of the 10 patients, only one survived. Femoral vein catheterization with unipuncture hemodialysis has markedly improved acute hemodialysis access. A video tape has been made of the technique and presented at the American Society of Artificial Internal Organs Meeting. Other areas of clinical investigation are also underway, with particular emphasis on pathophysiology of acute renal failure. The second course in Nephrology was sponsored by ISR Nephrologists and awarded credit by the American Medical Association for continuing education.</p>							

^a Available to Contractors upon originator's approval

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PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 68 AND 1498B 1 MAR 68 FOR ARMY USE ARE OBSOLETE

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

**REPORT TITLE: CLINICAL OPERATION, METABOLIC BRANCH, RENAL
SECTION, FOR TREATMENT OF SOLDIERS WITH RENAL
FAILURE**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 January 1975 - 31 December 1975

Investigators:

**William D. Myers, MD, Lieutenant Colonel, MC
Richard H. Merrill, MD, Lieutenant Colonel, MC**

Reports Control Symbol MEDDH-188 (R)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CLINICAL OPERATION, METABOLIC BRANCH, RENAL
SECTION, FOR TREATMENT OF SOLDIERS WITH RENAL
FAILURE

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 January 1975 - 31 December 1975

Investigators: William D. Myers, MD, Lieutenant Colonel, MC
Richard H. Merrill, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288 (R1)

The Renal Service performed 59 hemodialysis treatments, 567 urinalysis, 379 fibrinogen degradation product determinations, 13 creatinine clearance, and 9 glofil clearances. The glomerular histology protocol was extended to include scanning electronmicroscopy; a protocol was initiated to develop a noninvasive technique of determining intrarenal blood distribution; and another protocol was initiated to determine the site of infection within the urinary tract. The first annual Brooke Army Medical Center Nephrology Seminar was also held.

Renal Failure
Hemodialysis
Peritoneal Dialysis
Soldiers

CLINICAL OPERATION, METABOLIC BRANCH, RENAL SECTION FOR TREATMENT OF SOLDIERS WITH RENAL FAILURE

The Renal Section is composed of a Nephrologist, and two hemodialysis technicians who also function as research technicians. The primary mission of the Renal Section is to support the operation of the Clinical Division of the Institute of Surgical Research Burn Unit, providing both consultation for patients with renal and metabolic problems and hemodialysis in cases of renal failure. A secondary mission of the unit has been to support the Nephrology Service of Brooke Army Medical Center. The USAISR Hemodialysis Team provides backup support when necessary and assists in treatment of cases of renal failure occurring at the main hospital of Brooke Army Medical Center. The Chief of the Metabolic Branch directs the BAMC Nephrology Service, and the Chief of the Renal Section directly participates in Nephrology patient care at BAMC.

Ten patients were hemodialyzed for a total of 59 dialyses. One patient was peritoneally dialyzed. Of the 11 patients dialyzed only one survived. Ten patients died of burn wound sepsis. In addition to dialysis, renal technicians performed 567 urinalysis, 379 determinations of fibrinogen degradation products, 13 creatinine clearances, 9 glofil clearances, plus assisted the burn unit in bedside care.

The thrust of research by the Renal Section has been toward definition of the type of renal insufficiency seen in the thermally injured patient. Histologic studies including transmission electron microscopy have failed to support earlier suggestions that a glomerular alteration may be responsible for the altered renal function. This study has been extended to include scanning electron microscopy since current studies of other investigators have suggested a role of glomerular surface alteration in acute renal failure.

Efforts are also being made to better define the renal hemodynamics in the thermally injured patient. Currently a noninvasive technique for determining intrarenal blood distribution is being investigated in a rat model using dimercaptosuccinic acid labeled with Tc^{99m} and microspheres labeled with Sr^{90} .

Using fluorescein labeled immunoglobulin an investigation is being made into the role of retrograde urinary tract infection in renal parenchymal infection and systemic sepsis in the thermally injured patient. It would be particularly helpful if this technique could be used to localize sites of yeast infection since bladder irrigation would correct yeast infections limited to the lower tract without exposing patients to the toxicity of systemic therapy.

USAISR Nephrologists initiated the first Brooke Army Medical Center Nephrology Seminar, a three day event devoted to kidney transplantation held and videotaped at the Academy of Health Sciences. Speakers included Transplant Surgeons and Nephrologists of national status. The seminar was well received and future seminars are to be sanctioned by the American Medical Association and the American College of Physicians for continuing education. Chronic Renal Failure will be the topic of next years seminar.

PRESENTATIONS:

Merrill, RH: History of Transplantation First Annual Brooke Army Medical Center Nephrology Seminar 29-31 January 1975.

Myers, WD: Long Term Prognosis First Annual Brooke Army Medical Center Nephrology Seminar 29-31 January 1975.

Myers, WD: Principles of Acid-base Physiology ICU Advanced Nursing Course 19 Feb 75.

Myers WD: Flank Pain, Hematuria, and Renal Mass University of Texas Health Science Center at San Antonio combined Nephrology Conference 28 May 75

Myers WD: Principles of Acid-base Physiology ICU Advanced Nursing Course Aug 1975.

Merrill WH: Iron Absorption in Hemodialyzed Patients, Southeastern Dialysis and Transplantation Association Meeting, Dallas, Texas, August 1975.

Myers, WD: All About Kidney Transplantation ICU Advanced Nursing Course Seminar on Transplantation. October 1975

Myers WD: Treatment of Glomerulonephritis Brooke Army Medical Center. Selected Topics in Internal Medicine Lecture Series December 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)626	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61102A	3A161102B71R	01	084			
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a							
(U) Detection of Endotoxin in Burned Soldiers With Sepsis (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 03		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
Not Applicable				PRECEDING			
a. DATES/EFFECTIVE:		EXPIRATION:		76		.5	
b. NUMBER:				7T		17	
c. TYPE		d. AMOUNT:		CURRENT		.2	
e. KP & OF AWARD:		f. CUM. AMT.				5	
19. RESPONSIBLE GOV ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				Clinical Division			
				ADDRESS: Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: Robert B. Lindberg, PhD			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2018			
				SOCIAL SECURITY ACCOUNT NUMBER.			
21. GENERAL USE				22. ASSOCIATE INVESTIGATORS			
FOREIGN INTELLIGENCE NOT CONSIDERED				NAME: Virginia C. English, MS			
				NAME: Basil A. Pruitt, Jr, COL, MC			
				DA			
23. REFERENCES (Precede EACH with Security Classification Code)							
(U) Endotoxin; (U) Sepsis; (U) Assay; (U) Burns; (U) Humans							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
23. (U) Detect endotoxin in tissues of burn patients dying with sepsis; special interest directed toward liver, in view of current extensive findings of liver damage as part of pathological picture in septic shock, a factor in battle wounds of all types.							
24. (U) Liver and other tissues are triturated and extracted with glacial acetic acid to neutralize inhibition and permit detection of endotoxin.							
25. (U) 75 07 - 76 09 65 autopsies of patients dying with extensive burn injuries yielded liver-tissue for examination. 61 of these showed endotoxin by Limulus Amebocyte Lysate test. 93% of patients had detectable endotoxin in the liver; median value was .01 ug/gm. Normal livers failed to reveal endotoxin and bacterial content of reacting livers was 10 ³ or less. This level will not produce lysate coagulation.							

^a Available to contractors upon originator's approval

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE - SURGERY

REPORT TITLE: DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 June 1976

Investigators:

Robert B. Lindberg, PhD
Virginia C. English, MA
Arthur D. Mason, Jr, MD
Basil A. Pruitt, Jr, MD, Colonel, MC

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE - SURGERY

REPORT TITLE: DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 June 1976

Investigators: Robert B. Lindberg, PhD
Virginia C. English, MA
Arthur D. Mason, Jr, MD
Basil A. Pruitt, Jr, MD, Colonel, MC

Report Control Symbol MEDDH-288(R1)

Endotoxin can be demonstrated in plasma or serum by the Limulus amoebocyte lysate reaction, after extraction with acetic acid and neutralization of the supernate. A derivative of this procedure was applied to homogenates of liver from patients dying with severe burns, in most instances with clinical and laboratory indication of severe sepsis. 94% of 65 patients had a positive reaction for endotoxin in the liver. The median concentration was .044 mcg/gm of liver. Seven patients, or 11.4% of reactors, had no positive blood cultures. The sequestration is, in at least 50% of cases, not associated with bacterial contamination of the specimen; the remainder of reactors may include some in whom the bacterial contribution to endotoxin level occurred.

Endotoxin
Sepsis
Assay
Burns
Humans

DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

Detection of nanogram or lesser amounts of endotoxin (ET) is now feasible with the *Limulus amoebocyte* lysate reaction, and the availability of this technic has resulted in renewed interest in assessing the pathogenesis of gram-negative shock. Earlier reports have described the failure to detect circulating endotoxin in the blood of burned patients with sepsis (1). Those efforts led to the conclusion that circulating ET would be detected in approximately one third of the patients in whom gram-negative sepsis was plausibly established. The procedure thus is not a valid diagnostic aid in the diagnosis of sepsis, but other problems regarding ET and its mode of action can be approached by the LAL reaction. The study of liver tissue in patients dying with endotoxic shock was a problem to which LAL technic could be applied, and the results of this effort have been set down in this on-going study.

Since the endotoxin being detected with the LAL is a lipopolysaccharide embodied in the bacterial cell wall, bacteria in tissue could contribute a quantity of endotoxin directly, without representing any function of selective absorption of circulating endotoxin by hepatocytes, macrophages, or any other cell. This possibility was tested in several ways. One thousand bacterial cells in one ml of a broth culture will give a positive LAL reaction in some instances - i.e., when the cells are gram-negative bacilli or any other form rich in endotoxin. However, tests of liver tissues with varying numbers of *Enterobacteriaceae*, including *Klebsiella*, *Enterobacter* and *Serratia spp* have shown a wide variation in the minimum number of bacteria that will give a positive gelation with *Limulus* lysate. Positive reactions were obtained with a minimum of 5×10^3 cells per gram of liver tissue, but the median reactive level with 20 liver samples was 4.5×10^5 . In cultures, the reaction at 1000 cells per ml is the equivalent of .001 μ g or even .0001 μ g in standard endotoxin. Such a minimal reaction thus represents a lower cut-off point for interpreting the results of tests on clinical material: i.e., tissues with low bacterial counts and with ET levels at 10 to 1000 times the maximum that such bacterial level could produce may be interpreted as containing endotoxin beyond a level to be accounted for by bacterial content.

Endotoxin level was determined in 65 liver samples. Of these patients, 49 had at least one positive blood culture, and 16 had negative blood cultures or did not have a blood culture drawn. Results of ET determinations are shown in Table 1. There were 61 samples in which a detectable level of ET was found. Among these, 31, or 50% gave levels of .01 μ g/gm or higher. This meant a larger proportion than had previously been seen contained

1. Lindberg RB, English VC, Pruitt BA, Jr, Mason AD, Jr: Detection of endotoxin in burned soldiers with sepsis. USA Institute of Surg Res Ann Res Prog Rpt FY 1973, BAMC, Ft. Sam Houston, Texas, Section 6.

amounts of endotoxin which bordered on the levels which might reflect bacterial contamination rather than sequestered ET. However, the bacterial counts, shown in the third column range from high to low over the whole range of endotoxin levels observed. This supports the view that the bacterial count was not contributing consistently to the ET level demonstrated in these liver samples.

Table 1. Endotoxin Levels in Liver Tissues From
Burn Autopsies, ISR, 1975

Endotoxin ug/gm	No. Patients	Average Bacterial Count/gm
5.12*	1	$10^{6.7}$
.64	1	$10^{3.0}$
.32	3	$10^{2.8}$
.16	2	$10^{3.6}$
.08	4	$10^{3.1}$
.04	4	$10^{2.8}$
.02	8	$10^{3.4}$
.01	8	$10^{2.9}$
.008	1	$10^{3.0}$
.005	11	$10^{2.9}$
.0025	14	$10^{3.1}$
.001	4	$10^{2.3}$
<.0001	4	$10^{2.8}$
Total tested	65	
Median level of ET: .044 μ g/gm		
*This level was technically correct, but there is a possibility that direct fecal contamination of the sample occurred. It was omitted in calculating the median ET level.		

Endotoxin accumulated in livers of several patients who were clinically septic but in whom no positive blood cultures were collected. Table 2 presents data on patients in whom endotoxin was demonstrable and in whom all blood cultures were negative. There were seven patients in this category. Four had gram-negative bacteria in the liver. Multiple species were found in two, Enterobacter aerogenes in a third and Escherichia coli in a fourth. Three other liver samples were all negative for bacterial growth. Evidently the accumulation of endotoxin can occur in the absence of bacteria in the liver.

There were 4 patients in whom no detectable endotoxin could be found in the liver. One of these had no blood cultures collected. The other 3 had from 4 to 10 positive blood cultures. Multiple species were recovered from these samples. The results are shown in Table 3. The succession of species in blood cultures was not uncommon in a lethal sepsis. The blood stream strains were not always recovered from the liver in situations where sepsis had prevailed for a few days. Endotoxin was not detectable in any of these liver samples.

In autopsy liver samples positive for endotoxin, 12 species of bacteria were recovered. Predominant were Klebsiella pneumoniae, E. coli, Enterobacter cloacae and Pseudomonas aeruginosa. There was no indication that any species was associated with more ET in liver tissue than any other gram negative bacillus.

STABILITY OF LIMULUS AMOEBOCYTE LYSATE

A large pool of Limulus amoebocyte lysate was collected in 1971, and has been used since that time. The lysate, in 20 ml amounts, was frozen and stored at -70°C . Since the usefulness of ET assay depends on active lysate, periodic tests of potency of this lysate, have been carried out. It is of general interest to determine the duration of potency under these storage conditions. Results of a series of tests on samples from a pool of lysate designated 8-2a no.1 are summarized in Table 4.

In a series of 17 tests during 1975, variations in sensitivity occurred. A reading at 0.001 mcg/ml could be considered valid when the intensity of clot formed was rated at 2, which was a less than stable clot. However, only 4 out of 10 tests were read at this level. Ten tests gave 3 or 4 intensity reactions with a succession of endotoxin standards. Endotoxin standards themselves may vary; in the comparison series reported here, one control, in November 1975 gave the weakest results. That run gave a 2 reaction at 0.0025 $\mu\text{g/ml}$; at 0.00125 mcg. of ET of this preparation, the reaction was only at a weak clot level, grade 1 in this instance, the vial of lysate was regarded as sub-standard in potency, although other ET preparations reacted with stronger clots in this particular vial of lysate. The over-all result was affirmation of the survival of amoebocyte lysate activity for at least 4 years in storage at -70°C .

Table 2. Endotoxin in Livers of Patients with Negative
Blood Cultures, 1979

Autopsy	No. of Blood Cultures	Endotoxin Level mcg/gm	Liver	
			Bacterial Count per gm	Bacteria recovered in liver
6	1	.08	2×10^4	E coli, K pneumoniae, Ps aeruginosa, Prev stuartii, Pseudo maltophilia, Strep EpD
9	1	.02	1×10^2	Prev stuartii, K pneumoniae, Ps aeruginosa, Ps maltophilia
21	2	.04	1.2×10^4	Staph. aureus, E aerogenes, Strep EpD
38	12	.006	negative	no growth
39	10	.0025	negative	no growth
41	1	.02	5×10^4	E coli
64	4	.01	negative	no growth

Table 3
BLOOD CULTURE SURVEY OF PATIENTS WHO YIELDED
NO DETECTABLE ENDOTOXIN FROM AUTOPSY LIVER

AUTOPSY NUMBER	NO. NEG. AM. BLD. CULTURES	NUMBER OF BLOOD CULTURES AM YIELDING SPECIFIED ORGANISM	ORGANISMS FOUND IN LIVER
A-16-75	13	10 Positive bld. cultures giving rise to: 4 E. cloacae 4 P. aeruginosa 3 K. pneumoniae	COUNT: 2×10^2 E. aerogenes Nonhemolytic Gp D Streptococcus sp. K. pneumoniae
A-47-75	0	0	not examined
A-19-75	5	10. Positive bld. cultures giving rise to: 4 E. cloacae 2 P. aeruginosa 5 K. pneumoniae 1 S. aureus	1.1×10^3 K. pneumoniae S. aureus
A-11-75	6	4 Positive bld. cultures giving rise to: 3 E. cloacae 1 K. pneumoniae	5.1×10^1 P. aeruginosa K. pneumoniae

Abbr: No. = number; AM = antemortem; NEG. = negative; bld - blood

Table 4
THE INTEGRITY OF LIMULUS LYSATE STOCK

LYSATE U-2a # 1

DATE	DEGREE OF REACTION AT ET CONCENTRATION IN MCG.					
	0.01	0.005	0.0025	0.00125	0.0006	0.0003
1-14-75	3	3	2	2	1	1
Fresh endotoxin dilution from stock prepared for following tests:						
3-11-75	4	2	3	2	1	0
Fresh endotoxin dilution from stock prepared for following tests:						
7-22-75	4	3	3	3	3	2
7-23-75	4	3	3	3	3	2
7-29-75	4	3	3	3	2	1
Fresh endotoxin dilution from stock prepared for following tests:						
7-30-75	4	4	4	4	4	3
7-31-75	4	4	4	4	4	3
8-4-75	4	4	4	4	4	3
8-6-75	3	4	3	2	1	1
Fresh endotoxin dilution from stock prepared for following tests:						
8-11-75	4	4	3	3	2	1
8-25-75	4	4	4	4	3	3
Fresh endotoxin dilution from stock prepared for following tests:						
9-13-75	4	4	4	4	3	1
9-15-75	4	4	4	4	3	2
9-29-75	4	4	4	3	3	1
Fresh endotoxin dilution from stock prepared for following tests:						
11-10-75	4	4	3	2	+	+
New vial of lysate thawed and fresh endotoxin dilution prepared for following tests:						
11-28-75	4	3	2	1	1	1
1-5-76	4	3	2	1	0	0

Note: All lysate stored in Revco freezer at ~ 70 C & thawed before use.
0 = No reaction; 1 = positive but weak, unstable clot; 2 = slightly more stable clot than 1 +; 3 = Tight clot, but slightly disrupted on tipping tube; 4 = clot forms in less than 1 hour; does not disrupt on tipping tube.

All lysates assayed by using E. coli 0111:B4 lipopolysaccharide (Difco) as endotoxin source.

DISCUSSION

The observations summarized here add further evidence that endotoxin is indeed sequestered in the livers of burn patients dying with sepsis and gram negative infection. Although there were cases in the lower range (below .005 $\mu\text{g/ml}$) in which the bacteria present in the sample might have contributed to the endotoxin demonstrated, such samples were few in number. 94% of the patients exhibited demonstrable endotoxin in the liver. The mean value of .044 mcg/ml was at least 4 times higher than the level that might be caused by presence of gram negative bacteria at $10^4/\text{gm}$ of tissue.

The nature of the effect of endotoxin on hepatic cells remains to be elucidated. The complex derangement of essential functions that occurs with shock is such that this sequestering phenomenon may be an important factor in this aspect of burn injury.

PRESENTATIONS

Lindberg RB, English VE, Mason AD, Jr, Pruitt BA, Jr, "Sequestration of Endotoxin (ET) in Liver of Burn Patients with Gram-negative Sepsis" presented at the Am. Assoc. Immunol., Anaheim, Calif., April 13, 1976.

PUBLICATIONS

Lindberg RB, English VE, Mason AD, Jr, Pruitt BA, Jr: Sequestration of endotoxin in liver of burn patients with gram-negative sepsis. Fed Proc 35:738, 1976 (Abst).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&B(AR)636	
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23. (U) No single function is more important in microbiologic investigations in a burn unit than continued monitoring of infecting strains for antibiotic sensitivity. Resistance to antibiotics is often capricious--there is little predictability except by assessing the individual antibiotic. In military medicine or mass casualty management these predictions are of particular significance.							
24. (U) MIC by tube dilution is applied to appropriate, selected isolates, including all septicemic strains.							
25. (U) 75 07 - 76 09 Ninety-eight percent of strains tested were recovered from blood, most often from patients diagnosed as septic. Renewed methicillin resistance with staph aureus occurred after falling markedly in the preceding year. The newly epidemic strain of <u>Klebsiella pneumoniae</u> was affected only by Colymycin, Minocin and Vibramycin. This was time for epidemic <u>Enterobacter cloacae</u> as well. <u>Pseudomonas aeruginosa</u> was sensitive to Keflin and partly sensitive to gentamycin, but sensitive to carbenicillin.							

^a Available to contractors upon originator's approval.

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TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

**REPORT TITLE: ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN
PATIENT FLORA**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Daniel T. Zamora, SP6**

Reports Control Symbol MEDDH-288 (R1)

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ABSTRACT

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REPORT TITLE: ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN
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A high level of septicemia in severely burned patients presented 517 strains of essentially opportunistic invaders from blood cultures for sensitivity testing in 1975. MIC showed a return of methicillin resistance in a staphylococcus population that had in the previous two years become more sensitive to methicillin and other categories of antibiotics. For these strains, keflin susceptibility increased, clindamycin remained highly active, and they were all sensitive to vancomycin. Klebsiella pneumoniae, appearing on an epidemic scale to supplant Enterobacter cloacae, was inhibited in a significant degree only by colymycin, minocin and, in a preliminary trial, by vibramycin. Results were similar for Enterobacter cloacae. These enteric opportunists were major causes of sepsis, and essentially did not respond to chemotherapy. Together with Pseudomonas aeruginosa they represent a cross-resistant treatment-refractory bacterial population which appears most often in terminal septic episodes. Extended investigation of chemotherapy is called for if they are to be reduced in importance as a cause of death in severely burned patients.

Antibiotic sensitivity
Pseudomonas
Burns
Chemotherapy
Providencia

ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

Since bacterial sepsis has continued to be the major cause of death in burn injury, the antibiotic susceptibility of the burn flora in septicemia has remained a primary concern in the management of severe burns. The profile of sensitivity of those strains recovered in the Institute of Surgical Research from blood cultures, together with a small number of wound, sputum and urine cultures tested, furnishes a guide to current antibiotic therapy. Such treatment must frequently be instituted before the sensitivity of a given blood stream isolate could possibly be determined. A resume of sensitivity of the major offending species of bacteria enhances the potential for selecting the optimal antibiotic prior to acquisition of data on a specific strain. Bacteria found on burn populations may fluctuate rapidly in sensitivity to antibiotics so that ongoing summaries are important as guides in a valid program antibiotic therapy. The constitution of an optimal routine test battery of antibiotics was aided by sorting out long-term trends of sensitivity or resistance as seen in these summaries. Cross-resistance in several species of opportunistic pathogens found in burn patients at the Institute of Surgical Research has been far greater than that reported from other general hospitals. This difference permits more clearcut demonstrations of patterns of resistance than is the case with most organisms from hospital-acquired infections.

TECHNIC AND SOURCES OF STRAINS

In this study, a Minimum Inhibitory Concentration (MIC) procedure has been used. It has been described in a previous report. 10 mm capped plastic tubes are prepared to contain 1.0 ml of antibiotic at concentrations from 1.56 ug/ml to 50 ug/ml in Trypticase Soy Broth (TSB). 1.0 ml of a 4 to 6-hour TSB culture, containing from 10^4 to 10^5 organisms per ml is added to each tube of antibiotic and incubated for 18 to 20 hours. The final concentrations of antibiotic employed extended from 0.78 ug/ml to 25.0 ug/ml. MIC was taken as the lowest concentration of antibiotic which prevented visible growth at 18 to 20 hours. When needed, MIC could be determined by sub-culturing a loopful of fluid from tubes at concentrations above the visible growth level to blood agar plates.

Ten species of microorganisms were tested, with a total of 527 strains (Table 1). In previous years, up to 40% of strains examined were recovered from sources other than blood cultures, but in 1975, 519 out of 527 strains (98%) were from blood cultures. Thus, the strains examined in the period covered by this report are those which, on an empirical basis, were most

1. Lindberg RB, Contreras AA, Smith HOD, Jr, Plowey EC, Mason AD, Jr: Antibiotic sensitivity of flora from military burn patients. USA Inst Surg Res Progress Rpt FY 1973, BAMC, Fort Sam Houston, Texas. Section 7.

probably virulent, since they had invaded the blood stream. The data collected are, of course, of maximum pertinence to the essential question of the control of sepsis in burned patients.

The species most frequently encountered in blood cultures were, in order of occurrence, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Enterobacter cloacae. In previous reports, Providencia stuartii had been found to be a major cause of sepsis in burned patients at the Institute of Surgical Research. From 1970 through early 1974, it rose from being a conspicuous opportunistic invader to the predominant species numerically; however, in 1975, only one strain was recovered from a blood culture.

Table 1. Sources and Species of Bacteria Tested for Antibiotic MIC, 1975

Species	Total No. Tested		Blood	Sources of Strains		
	Patients	Strains		Lung	Wound	Other
<u>S. aureus</u>	43	153	152	1	0	0
<u>S. epidermidis</u>	6	6	6	0	0	0
<u>Strep. spp. (alpha, non-hemolytic)</u>	7	13	13	0	0	0
<u>Ps. aeruginosa</u>	45	95	91	2	1	1
<u>K. pneumoniae</u>	58	159	156	2	0	1
<u>Entero. cloacae</u>	31	62	62	0	0	0
<u>E. coli</u>	8	9	8	1	0	0
<u>S. marcescens</u>	7	22	21	1	0	0
<u>Prot. mirabilis</u>	4	7	7	0	0	0
<u>Prov. stuartii</u>	1	1	1	0	0	0
Totals		527	517	7	1	2

The antibiotics used in the test battery were selected in accordance with current trends in the field of antimicrobial usage, and also with reference to the effectiveness displayed in vitro against this particular bacterial population. The antibiotic battery used in 1975 is shown in Table 2.

2. Lindberg RB, et al: Providencia stuartii: A new opportunistic pathogen recovered from burns. In Research in Burns: Proceedings of 4th International Congress on Research in Burns. (in press).

Table 2. Antibiotics Used in Testing Gram-Positive and Gram-Negative Bacteria in 1975

<u>Gram-Positive</u>	<u>Gram-Negative</u>
Gentamicin	Gentamicin
Linco [*]	Minocin
Methicillin	Kanamycin
Oxacillin	Ampicillin [*]
Nafcillin	Keflin
Vancomycin ^{**}	Colymycin
Keflin	Tobramycin ^{**}
Minocin	
Clindamycin	
Tobramycin ^{**}	
Vibramycin ^{**}	

* Lincocin was deleted in mid-1975; Ampicillin was deleted in October 1975.

** Vancomycin was introduced in August 1975; Tobramycin was introduced in November 1975; Vibramycin was introduced in mid-1975.

RESULTS OF SENSITIVITY TESTS ON PRINCIPAL SPECIES OF BURN FLORA BACTERIA

Sensitivity of bacterial populations tested in this series are summarized in the following tables. Results are shown in cumulative increments, so that each increment of antibiotic inhibits all strains that were inhibited by lesser amounts. Although the most precise assessment of sensitivity in relation to achievable effective blood level varies with each antibiotic, an approximation of sensitivity demarcation level may be indicated at 6.5 ug/ml for gram-positive organisms and 12.5 ug/ml for gram-negative bacilli.³

Staphylococcus aureus - The cumulative sensitivity of 153 strains of S. aureus tested in 1975 is shown in Table 3. The group of methicillin-type antibiotics has been of particular interest due to the extremely high incidence of methicillin-resistance which was present in staphylococci from 1969 to 1973. In that year, sensitive strains increased in incidence, and sensitivity rose still further in 1974, but in 1975, resistant forms reappeared; only 21.8% of isolates were inhibited at 6.25 ug/ml of methicillin. Gentamicin was lower in inhibitory activity than in the previous year, and a marked decrease also occurred with minocin. The most effective antibiotic in the test

3. Finland M. Changing patterns of susceptibility of common bacterial pathogens to antimicrobial agents. Ann Int Med 76: 1009, 1972.

Table 3. Cumulative Inhibitory Levels for 153 Strains of Staphylococcus aureus - ISR, 1975

Antibiotic ug/ml	Antibiotic and % of Strains Inhibited at Each Level										
	G	L*	Sc	Ps	U	Va**	Kf	M	Cl	To**	Vb**
> 25	100	100	100	100	100	100	100	100	100	100	100
25	93.2	96.6	88.0	93.0	96.4	100	99.3	93.0	98.6	88.8	100
12.5	79.0	96.6	46.3	85.4	93.4	100	98.6	69.4	98.6	88.8	100
6.25	38.8	96.6	21.8	73.6	85.6	100	97.2	46.5	98.0	88.8	78.3
3.12	24.3	72.8	10.5	52.0	71.2	98.8	89.7	34.7	95.3	55.5	26.4
1.56	18.2	47.4	0	27.0	46.4	81.6	76.7	28.4	94.7	11.1	19.8
< 0.78	5.4	13.5	0	6.9	22.8	4.5	52.7	23.6	88.0	0	18.8
Total Tested	148	59	151	144	153	87	146	144	151	9	106
No. of patients on whom strains were collected: 59											

C: Gentamicin; L: Lincocin; Sc: Methicillin (Staphcillin); Ps: Oxacillin (Proctaphlin);

U: Nafcillin (Unipen); Va: Vancomycin; Kf: Cephalothin (Keflin); M: Minocin; Cl: Clindamycin;

To: Tobramycin; Vb: Vibramycin.

* Lincocin discontinued in test battery: 1975

** Vancomycin, Tobramycin and Vibramycin added to test battery: 1975

battery was vancomycin; this drug was first tried in 1975. All but one of 87 strains tested were inhibited at 3.12 ug/ml. Keflin was similarly effective, as was clindamycin. Lincocin was deleted from testing during 1975, although it was still effective in vitro. Tobramycin was tested late in 1975; it has thus far been highly active. There were no instances of complete cross-resistance in this group of strains, although cross-resistance was common.

Despite this encouraging proportion of sensitive strains, success in treating staphylococcal sepsis was not great. Vancomycin was selected as the drug of choice for clearing the blood stream in staphylococemia, in hopes of preventing the development of endocarditis.

Retrospective comparison over a period of several years offers a major insight on patterns of resistance and susceptibility of staphylococci to antibiotics. The data for the period from 1968 through 1975 are summarized in Table 4. Strains inhibited at 6.25 ug/ml are regarded as sensitive; above that level, , resistant. The progression of increasing methicillin-resistance in the period from 1969 to 1972 is dramatically shown. No comparable incidence of methicillin-resistance has been found in other descriptions of this phenomenon. The re-appearance of a sensitive population in 1973 was described in a previous report; however, the reemergence of resistant strains in 1975 was a new episode, which resembled the earlier experience. The linkage of resistance to methicillin to the aminoglycoside antibiotics was suggested by the progression of events with gentamicin. This was a less consistent fluctuation, but the 8 year experience encompassed the extremes between 32% of strains sensitive and 92% sensitive. After 2 years of increasing effectiveness (1973-1974) which paralleled the behavior of methicillin, the strains recovered in 1975 shifted abruptly to a low level of gentamicin sensitivity. Minocin behaved in a similar manner. Other effective antibiotics have not been in use long enough for similar observations to be made.

Staphylococcus epidermidis - Only 6 strains of S. epidermidis were recovered from blood cultures, each in a single positive culture. Two of the 6 patients died, but neither of these was classified as death due to S. epidermidis. Sensitivity varied widely, but from half to two-thirds of the 6 strains were sensitive to the antibiotics used with gram-positive cocci. The highest degree of sensitivity - less than 0.78 ug/ml - was observed with gentamicin, keflin, minocin and clindamycin.

Alpha and non-hemolytic Streptococci - A small but significant number of strains of alpha-hemolytic and non-hemolytic streptococci have been recovered from blood cultures annually. In 1975, 13 strains of streptococci were recovered from 7 different patients. Five of these patients died. The sensitivity of these 13 strains is summarized in Table 5. Most strains were resistant to gentamicin, and all were methicillin and oxacillin resistant. Keflin was virtually ineffective, as was clindamycin. Penicillin G was the most effective antibiotic tested, and half of the strains were sensitive to minocin. This heterogeneous population of streptococci were mainly group D by biotype. Although they were numerically inconspicuous, their minimal spectrum of antibiotic sensitivity indicates that their presence in burn patients with sepsis presents a potentially

Table 4. Comparative Sensitivity of *Staphylococcus aureus* on an Annual Basis, ISR - 1968-1975

Antibiotic	Year and % of Strains Inhibited at 6.25 ug/ml of Antibiotic							
	1968	1969	1970	1971	1972	1973	1974	1975
Gentamicin	-	52.0	32.0	50.0	35.0	67.9	92.2	38.8
Lincocin	64.7	48.5	29.8	28.4	26.0	44.3	93.9	96.6
Methicillin	84.6	25.7	18.0	15.5	13.1	50.0	65.2	21.8
Oxacillin	80.0	33.0	22.4	20.1	18.8	69.7	82.6	73.6
Nafcillin	90.0	41.0	33.9	33.0	26.0	62.3	83.3	85.6
Vancomycin	-	-	-	-	-	-	-	100.0
Keflin	-	-	-	56.4	22.6	72.1	90.4	97.2
Minocin	-	-	-	-	-	84.1	96.0	46.5
Clindamycin	-	-	-	-	-	40.7	95.8	98.0
Tobramycin	-	-	-	-	-	-	-	88.8
Vibramycin	-	-	-	-	-	-	-	78.3

Table 5. Alpha and Non-hemolytic Streptococci Sensitivity
of 13 Strains from Blood Cultures in Burned Patients, ISR
1975

Concentration ug/ml	G	Antibiotic and % of Strains Inhibited							Pen C
		L	Sc	Ps	U	Kf	M	Cl	
25	100	100	100	100	100	100	100	100	100
25	23.0	41.6	7.6	84.6	100	69.2	92.3	92.3	100
12.5	23.0	8.3	0	20.7	100	23.0	76.0	69.2	100
<hr/>									
6.25	23.0	0	0	0	69.2	7.6	53.8	23.0	100
3.12	15.3	0	0	0	15.3	0	46.1	7.6	83.3
1.56	15.3	0	0	0	15.3	0	46.1	7.6	0
0.78	15.3	0	0	0	0	0	46.1	7.6	0
Total Tested	13	12	13	13	13	13	13	13	6

intractable infection as a possibility.

Pseudomonas aeruginosa - Although Ps aeruginosa has been essentially controlled since 1964 by use of appropriate antibacterial topical therapy, it remains a conspicuous part of the burn wound flora, and was third in frequency of species recovered from septicemia in burn patients in 1975. Many of these septicemic episodes can be classified as terminal events, but even so, the species can scarcely be described as controlled in this burn population. Ps. aeruginosa has been frequently found to be resistant to most antibiotics, and in the ISR experience, this is indeed the situation. Results of sensitivity tests with strains from 45 patients with positive Pseudomonas blood cultures are shown in Table 6. Gentamicin-resistant strains were in the majority; minocin was effective in a small percentage of strains but keflin, ampicillin and kanamycin were completely ineffective. Colymycin remained the most effective antibiotic in vitro. Disappointingly, tobramycin and vibramycin were minimally effective. Carbenicillin constituted a special case, since inhibition at high levels of antibiotic still left it acceptable for use. At 156 ug/ml, 88% of strains were inhibited. Two experimental antibiotics, ticarcillin and amikin, were tested extensively in 1974; these drugs were not available for further test in 1975 due to administrative procedures in securing approval of these agents. Both were highly effective in vitro against Ps. aeruginosa. No other antibiotics tested have shown such a level of inhibitory action.

On comparing sensitivity of Ps. aeruginosa in 1975 with results reported in previous years, only one significant change in proportion of sensitivity was found: a drop in proportion of strains sensitive to gentamicin. During 1969-1974, from 62% to 84% of isolates were sensitive to gentamicin; in 1975, only 40% of isolates were sensitive. During the same period, sensitivity to carbenicillin increased gradually from a low of 30% of strains tested in 1971 to 88% in 1975.

Klebsiella pneumoniae - Among Enterobacteriaceae species, K. pneumoniae was most commonly encountered in septicemia in 1975. This constituted a marked change in identity of opportunistic invading bacteria since 1974, when Enterobacter cloacae was a major cause of septicemia. The source of Klebsiella spp is, first of all, endogenous, since they are ubiquitous in the gut. The uniformity of biotype and antibiogram in strains recovered during 1975, however, suggested that a small number of epidemic strains may be represented, with patient to patient transfer constituting the infection source.

Cumulative sensitivity for 159 strains K. pneumoniae from 58 patients with septicemia is summarized in Table 7. The susceptibility of spectrum was not encouraging. Minocin was effective with half the strains; colymycin was active with 73% of strains tested and vibramycin, tested here for the first time, was the only other effective antibiotic. The well-established phenomenon of resistance transfer factor is undoubtedly involved in this increasingly numerous opportunistic pathogen and selective enhancement of a resistant population tends to occur.

Table 6. Pseudomonas aeruginosa Cumulative Inhibitory Levels for 56 Strains
ISR - 1975

Concentration g/ml	G	M	Antibiotic and % of Strains Inhibited					To*	Vb*	Conc. ug/ml	Cb
			K	Amp	Kf	Co					
>25	100	100	100	100	100	100	100	100	100	1250	100
25	44.2	35.7	4.1	0	0	87.3	18.5	100	100	1250	98.7
12.5	40.0	16.8	0	0	0	86.3	18.5	20	625	93.8	
6.25	29.4	4.2	0	0	0	84.2	18.5	10	312	92.5	
3.12	23.1	2.1	0	0	0	72.6	14.8	0	156	68.8	
1.56	9.4	2.1	0	0	0	35.7	11.1	0	78	81.4	
<0.78	1.0	1.0	0	0	0	10.5	3.7	0	39	50.6	
									19	9.8	
No. Tested	95	95	96	86	96	95	27	10	9	2.4	Total 81

G: Gentamicin; M: Minocin; K: Kanamycin; Amp: Ampicillin; Kf: Keflin; Co: Colymycin; To: Tobramycin
Vb: Vibramycin; Cb: Carbenicillin (tested up to 1250 ug/ml).

* Tobramycin and Vibramycin tested in the last 3 months of 1975 at the time when ampicillin was deleted from the test battery.

Table 7. Klebsiella pneumoniae: Cumulative Sensitivity for 159 Strains
ISR - 1975

Concentration ug/ml	G	M	Antibiotic and % of Strains Inhibited					To	Vb
			K	Amp	Kf	Co			
25	100	100	100	100	100	100	100	100	
25	10.8	81.2	5.6	4.7	26.5	73.2	13.2	100	

12.5	6.3	52.2	5.6	3.9	22.1	73.2	9.6	94.4	
6.25	3.8	21.9	3.7	3.9	7.5	72.6	9.6	88.8	
3.12	3.8	12.2	0.6	3.9	3.7	71.9	9.6	88.8	
1.56	0	6.4	0	0.7	0.6	63.0	2.4	58.3	
< 0.78	0	0	0	0	0	46.4	0	0	

Total Tested	157	155	159	126	158	157	83	36	

In view of the predominance of K. pneumoniae in sepsis in 1975, a retrospective comparison of sensitivity of strains collected from sepsis in ISR patients is appropriate. Table 8 presents this data. Prior to 1974 numerous strains from sputum were tested; in 1974 and 1975, Klebsiella septicemia became far more frequent in occurrence and only blood stream isolates were tested. Sensitivity to Kantrex occurred at the 30% level until 1973, when a sharp increase in sensitivity occurred, but when Klebsiella septicemia strains increased in 1974, sensitivity to Kantrex abruptly fell to less than the 5 to 6% level. Keflin was consistent in inhibiting only about one-fifth of the strains tested, with the exception of the 1973 group. Change in sensitivity to gentamicin was especially disturbing; this useful aminoglycoside, which had been the most effective antibiotic prior to 1974, became virtually ineffective by the time the 1975 collection was tested. An inversion of effectiveness of colymycin occurred in the same interval; this antibiotic was much more effective after 1974 than it had been previously. Of 2 new antibiotics, vibramycin has been highly effective in vitro; tobramycin was virtually inactive. The loss of sensitivity that accompanied a marked rise in frequency of Klebsiella sepsis strongly suggests the incursion of an epidemic strain or strains which essentially reside in the burn ward, but this is, at present, only an hypothesis. Further study of these epidemic isolates is being carried on.

Table 8. Comparison of Antibiotic Sensitivity
of Strains of Klebsiella pneumoniae from Burned Patients
ISR - 1971-1975

Antibiotic	Year and % of Strains Sensitive				
	1971	1972	1973	1974	1975
Kantrex	31.2	32.5	72	8.2	5.6
Keflin	24.1	18.6	60.8	13.8	22.1
Gentamicin	71.8	60.4	83.3	15.2	6.3
Colymycin	31.2	48.8	32	95.8	73.2
Ampicillin	0	4.6	8	1.3	3.9
Minocin	NT*	NT	83.3	90.4	52.2
Vibramycin	NT	NT	NT	NT	94.4
Tobramycin	NT	NT	NT	NT	9.6
Total Strains Tested	32	43	25	73	159

* Not tested

Enterobacter cloacae - In the previous year, 1974, Enter. cloacae increased abruptly in incidence in septicemia and became the predominant species of gram-negative bacillus in the blood of patients who developed sepsis. This incidence decreased abruptly after April 1975, and since June 1975, Enter. cloacae sepsis has been seen infrequently. Sixty-two

strains from blood cultures on 31 patients were tested in 1975, and their antibiotic sensitivity is summarized in Table 9. The strains were broadly resistant to antibiotic; only minocin and colymycin were effective at 12.5 ug/ml with 75% of strains tested. The organisms were resistant to gentamicin, ampicillin, kanamycin and keflin. Only 6 strains were tested with vibramycin; its encouraging result will require further confirmation, as will the negative results with 6 strains tested with tobramycin.

The behavior of this epidemic outbreak of a common enteric species resembles the experience of previous years with Providencia stuartii. In such instance a species with no history of causing severe invasive sepsis in burn patients appeared in the burn ward and rapidly assumed a state of predominance. Enterocloacae was not quite as resistant to antibiotics as was Providencia, but the strains were sufficiently resistant to present a major therapeutic problem when they invaded the burn patient. Although the chronologic sequence of events was consistent with the presence of a continually transmitted opportunistic strain in the burn ward, the same species was recovered from several patients at the time of their arrival at the Institute of Surgical Research, and the species was recognized in at least 2 other burn research centers. Available data does not permit categorization of the outbreak as a self-contained epidemic in a single burn ward, although patient-to-patient transmission certainly occurred extensively.

The evident cessation of the Enterobacter sepsis outbreak has no ready explanation. No control measures had been instituted prior to its sudden drop in incidence, nor did the strain actually disappear. In the first half of 1975, 22 patients showed Enterocloacae in one or more blood cultures. From June to December, only 9 patients had positive blood cultures with this species. Since 1975, Enterocloacae has been recovered but rarely from wounds, sputum, or blood. This episode illustrates a basic pattern of opportunistic epidemic infection in burn wards; whether the capability of setting up such epidemics is species-related may be determined with continued systematic monitoring of the burn population.

Infrequent Enterobacteriaceae - Escherichia, Serratia, Proteus and Providencia - Species of Enterobacteriaceae less frequently encountered are summarized in Table 10. Escherichia coli and Serratia marcescens were involved in 8 and 7 patients respectively. The organisms were not benign; 5 patients, who exhibited E. coli septicemia, died, as did 6 out of 7 patients with Serratia sepsis. Gentamicin, keflin, and especially colymycin were the most effective antibiotics for E. coli. Serratia strains were totally and unequivocally resistant to the established test battery of antibiotics. Vibramycin was available only for testing of 5 strains; 3 of these were sensitive to 12.5 ug/ml. It is fortunate that this highly resistant species has not shown further epidemic spread; however, the propensity of Serratia for causing nosocomial infections has been documented on numerous occasions in different hospitals, and its behavior in the ISR burn population will continue to be observed in detail.

DISCUSSION

Table 9. Enterobacter cloacae: Cumulative Sensitivity of 62 Strains
Recovered from Blood Cultures, ISR - 1975

Concentration ug/ml	G	M	K	Antibiotic and % of Strains Inhibited				To	Vb
				Amp	Kf	Co			
> 25	100	100	100	100	100	100		100	100
25	100	91.9	8.0	5.3	4.8	82.2		33.3	100
12.5	8.3	75.8	8.0	3.5	0	77.4		16.6	100
6.25	6.6	61.2	4.9	0	0	75.8		16.6	100
3.12	3.3	24.1	0	0	0	74.1		16.6	66.6
1.56	3.3	6.4	0	0	0	74.1		0	50.0
< 0.78	0	0	0	0	0	46.7		0	0
Total Tested	60	62	61	56	62	62		6	6

Table 10. Infrequent Enterobacteriaceae in Burn Sepsis:
Escherichia, Serratia, Proteus, Providencia

Species	No. Patients Positive	No. Strains	C	Antibiotic and % Inhibited at 12.5 ug/ml						Vb
				M	K	Amp	Kf	Co	To	
<i>Escherichia coli</i>	8	9	55	22	22	28	55	100	33	33
<i>Serratia marcescens</i>	7	22	0	0	0	0	0	0	0	60
<i>Proteus mirabilis</i>	4	7	50	0	28	43	59	0	0	0
<i>Providencia stuartii</i>	1	1	0	0	0	0	0	0	0	0

* Only 5 strains of *Serratia* tested with vibramycin.

The most significant species involved in bacterial sepsis in burned patients in 1975 were S. aureus, K. pneumoniae, Ps. aeruginosa and Entero. cloacae. When septicemia occurred in burn patients with these or with any of the 7 other species of bacteria recovered from the blood in 1975, a relatively high mortality could be predicted, even when an antibiotic active against the offending organism could be found. A retrospective survey of the type set down here aids in selecting the most promising antibiotic at the first indication of invasive infection. STaphylococci, having gone through a remarkably high level of methicillin resistance, regained up to a 68% sensitivity level during 1973 and 1974, only to have methicillin-resistance reappear. The high level of sensitivity to keflin and vancomycin indicated that they are the most promising antibiotics for use at this time. These fluctuations in sensitivity of a microbial species to antibiotics appeared to be unrelated to specific changes in antibiotic usage.

The increase of incidence of K. pneumoniae made this opportunistic invader the principal pathogen in septicemia in 1975. A progressive increase in resistance of Klebsiella to a broad spectrum of antibiotics has occurred during the past 5 years. Kantrex and gentamicin have virtually lost effect on K. pneumoniae; keflin has, during this time, been effective only with 20% of isolates, and colymycin, present the most effective antibiotic, has occupied this status only during the past 2 years. An encouraging pattern appeared with vibramycin, which in a small series tested was highly active. The ease with which resistance factors are acquired by Klebsiella, and its capacity for intensive colonization of burn patients, militate against complacency regarding any antibiotic as being a definitive solution for control of Klebsiella in the burn ward. In 1974, an experimental antibiotic, amikacin (Bristol Laboratories), was tested against gram-negative strains pertinent to the burn wound. It inhibited 75% of strains of Klebsiella at 3.12 ug/ml and was obviously a promising drug for use against such infections. It is to be released for use in 1976.

Entero. cloacae exhibited a sensitivity pattern closely paralleling Klebsiella in 1975. Minocin was more effective against this species than against Klebsiella, but, as with that genus, colymycin was the only other antibiotic in general use that was active against Enterobacter. Vibramycin was completely inhibiting in a preliminary trial at the 6.25 ug/ml level.

Ps. aeruginosa, despite the long history of successful control of surface infection by topical therapy, remains a major part of the flora in sepsis in burns. In 1975, the strains recovered from septicemia were somewhat more resistant to antibiotic than they had been earlier. Gentamicin resistance increased in 1975; only 40% of the strains tested were inhibited at the 12.5 ug/ml level. Colymycin remained an effective antibiotic in vitro; and carbenicillin was slightly more effective in 1975 than it had previously been.

The armamentarium of antibiotics available for control of gram-negative sepsis in burns remained disturbingly limited in terms of predominant organisms in 1975. Newly developed agents, such as amikacin and vibramycin merit prompt, careful consideration if this life-threatening problem is to be better controlled.

PRESENTATIONS

Lindberg RB: Epidemic methicillin-resistant Staphylococcus aureus type 84 in a burn unit. Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, DC October 11, 1975.

Lindberg RB: Bacteriologic and clinical aspects of methicillin-resistant staphylococcus epidemics in a burn unit since 1968. 8th Annual meeting, American Burn Association, San Antonio, Texas, April 2, 1976.

PUBLICATIONS

None

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23. (U) Continued high level of <u>Staphylococcus aureus</u> septicemia persisted despite intensive use of antimicrobial therapy. A persisting monotype epidemic, with recurring methicillin resistance, required characterizing as a valuable model for understanding nosocomial outbreaks and the possibility of controlling them, a major objective in military medicine.							
24. (U) Staph phage typing, MIC sensitivity testing and antibiograms were used in epidemic tracing.							
25. (U) 75 07 - 76 09 Continued predominance of phage type 84 in epidemic pattern in burn patients occurred despite a reappearance of methicillin sensitive strains in the burn ward. In 1975, a reappearance of methicillin resistance took place. Cephalosporium and glycopeptides especially Vancomycin, remained relatively effective in controlling staphylococcal sepsis. The variability of staphylococcal populations required continued monitoring in view of the unusual fluctuations described.							

^a Available to contractors upon originator's approval

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

**REPORT TITLE: EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS
AUREUS TYPE 84 AND 84,85 IN BURNED MILITARY PERSONNEL**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 June 1976

Investigators:

**Robert B. Lindberg, PhD
Ruth L. Latta, BS
Basil A. Pruitt, Jr, MD, Colonel, MC
Arthur D. Mason, Jr, MD**

Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS
AUREUS TYPE 84 AND 84,85 IN BURNED MILITARY PERSONNEL

US Army Institute of Surgical Research Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 20 June 1976

Investigators: Robert B. Lindberg, PhD
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Phage typing and MIC sensitivity testing were used to characterize the Staphylococcus aureus found extensively in burn patients. A previous epidemic of Phage type 84 was supplanted in mid-1974 by type 84,85. Extremely high methicillin-resistance was observed, subsiding abruptly after several years, only to re-emerge in 1975. Cross-resistance was observed with cephalothins, and with aminoglycosides as well. Staphylococcal seeding of the burn wound is an obvious source of propagation, and selective pressures, endogenous and exogenous, contribute to the continued persistence of this ubiquitous species. Currently adequate in vitro sensitivity prevails, but the possibility of re-emergence of resistance factors is shown by this study.

Staphylococcus
Burns
Septicemia
Burn Infections
Humans

EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE 84 AND 84,84 IN BURNED MILITARY PERSONNEL

The continuing problem of emergence of antibiotic resistant *Staphylococci* in hospital populations has prompted detailed study of the behavior of *Staphylococcus aureus* in the patient population of ISR. The organism is ubiquitous, and eradication of seeding by *Staph aureus* is an unrealistic goal. Control of infection by antibiotic remains the only feasible approach to prevention of staphylococcal sepsis in severe burns. In previous reports (1) description of the appearance of a uniquely methicillin-resistant population of staphylococci was described. Fluctuations of sensitivity were observed over several years, and in 1973 and 1974, susceptible strains reappeared after a 4-year period of maximum methicillin-resistance. However, these organisms do not of necessity remain static, and once more, methicillin-resistance was on the rise in 1975. The necessity for continuing close observation of this major pathogenic species in burn patients is only too obvious.

Phage Types of *S. aureus* Over the Period 1968-1975

The incidence of predominant staphylococcal phage types recovered from burn patients in the ISR, from 1968 through 1975, is outlined in Figure 1. The types listed are, of course, not the total number of phage types observed, but they are the major ones that have been identified. The percentage of patients harboring a given phage type is shown as the fraction of staphylococcus positive patients observed; it includes the entire block outline. The solid black area is the proportion of all strains of staphylococci reacting in that type identity for that year.

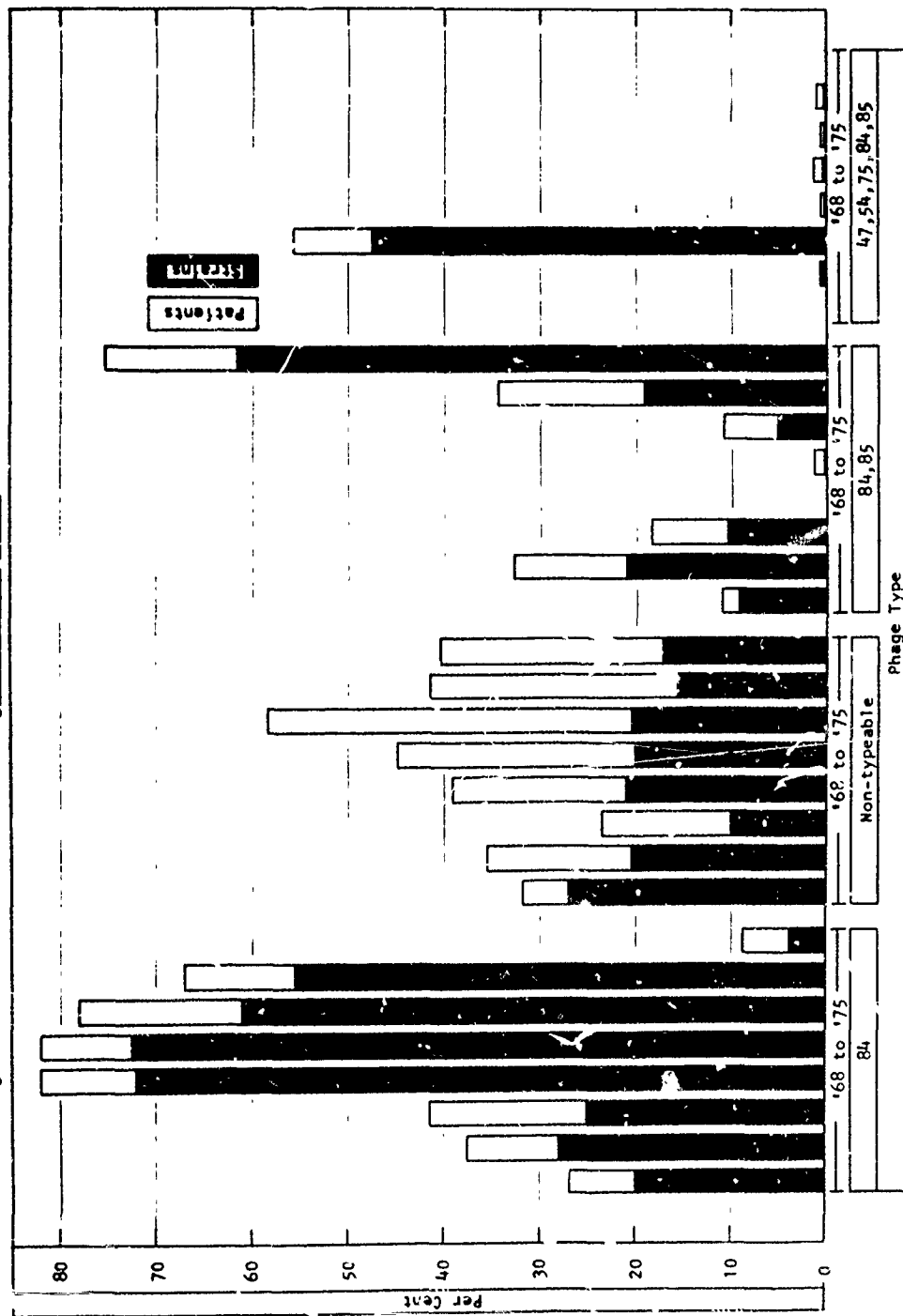
Type 84 was present from 1968 to 1970, but was a relative minority of the strains typed. It became the predominant type in 1971, and continued in that status through 1974. In the last year, 1975, a striking drop in its incidence occurred. From a status of recovery of type 84 in over 80% of patients in 1971 and 1973, it fell to 67% of patients in 1974; then in 1975, it was found in only 9% of patients.

Type 47, 54, 75, 84, 85 is presented because of its part in the continuing rise and disappearance of phage types in the burn patients. In 1970 it was the predominant form, but in 1971, it disappeared almost completely. A small number of strains were recovered through 1974, but in 1975, none were found.

The close relationship of type 84 with 84,85 has been noted in earlier reports. 84,85 was found in numbers comparable to type 84 in 1968-1970; it then fell to negligible numbers until 1974, when it was found in 34% of patients. In 1975, however, it supplanted type 84 as the predominant type; this transition was abrupt, and unprecedented.

1. Lindberg RB, Latta RL, Pruitt BA, Jr, Mason AD, Jr: Emergence of methicillin-resistant *Staphylococcus aureus* Type 84 and 84,85 in Burned Military Personnel. US Army Ann Historical Rept 1976: pp93-105.

Figure 1. Incidence of Predominant *Staphylococcus aureus* Phage Types, 1968 - 1975



Non-typable strains fluctuated in incidence but not to an extent that suggested any marked invasion of a strain not typable by the current typing set. The relatively large proportion (up to 56% of patients harboring non-typables in 1973) with the low proportion of all isolates in this category reflects the fact that any patient may harbor non typable strains but they are seldom predominant in the patient population.

S. aureus Phage Types in the ISR Burn Ward, 1975

Admissions to the ISR totalled 245 patients in 1975. Among these, 135 patients contributed staphylococci from various sources, and 14 patients admitted in late 1974 were also sources of isolates. 844 strains were thus collected from 149 patients. Sources included wounds, sputum, biopsy, catheter tips, urine, and other sites, plus autopsy tissues. Phage types observed, by patient and in proportion of strains, are shown in Table 1. This was the first time that type 84,85 had predominated since these phages were included in the typing set. It had appeared in 1974, but was found only in half as many patients as harbored type 84. The drop in occurrence of type 84 was marked and dramatic; only 8.7% of patients harbored this type in 1975. Non-typable (NT) strains were found in 40.3% of patients; this number has been even higher in some years, but the proportion of total strains that are NT is always low.

Phage type 94, formerly designated as WH-1, reached a significant level of incidence for the first time 1974, when 10.2% of patients harbored it. This proportion rose to 13.4% in 1975, and more significantly, the proportion of total strains of type 94 rose from 2.3% to 9.5%. Although this strain was not a major problem, it merits close observation since the sequence of increasing incidence resembles that which has been seen with other types in former years.

Table 1. Predominant Phage Types of Staphylococcus aureus Strains from 149 ISR Burn Ward Patients, 1975

Phage Type	Patients	Strains
	Per Cent	
84,85	75.2	61.3
Non-typeable	40.3	16.9
94	13.4	9.5
84	8.7	3.8
29	3.4	0.9
85		0.8
83A,85	2.7	0.5

Types 29, 85, and 83A,85 were found in very small numbers. Their presence confirmed the versatility of the typing system. The total of typed stains and unequivocal NT strains made up 93.7% of all 844 strains collected.

S. aureus in Septicemia, ISR, 1975

The epidemiologic pattern of staphylococcal septicemia and especially of endocarditis and thrombophlebitis in ISR patients suggests, on the basis of clusters of episodes, that specific strains of staphylococci may at times set up circumscribed local epidemics. One clue to the validity of this hypothesis would be differences in incidence of specific phage types from septic episodes in contrast to the overall population of staphylococci. The bacteriologic aspect of septicemia was scrutinized with this possibility in mind. There were 44 patients with staphylococemia in 1975, and 142 strains of S. aureus were recovered in blood cultures.

The order of frequency with which phage types were recognized is shown in Table 2. Phage type 84,85 was overwhelmingly preponderant; its incidence closely paralleled that found in the total staphylococcus population. In 1974, it was recovered from 31% of patients with staphylococemia. The proportion of non-typable strains decreased from the level which was found in 1974. Only 9.1% of patients had non-typable strains in blood culture. In contrast to the 6.1% of patients harboring such strains in 1975, the low level of NT strains in blood in 1975 suggests that this specific type 84,85 exhibited selective ability to invade the blood stream.

Table 2. Phage Types of Staphylococcus aureus Strains from Blood Stream of 44 ISR Burn Ward Patients, 1975

Phage Type	Patients	Strains
	Per Cent	
84,85	81.8	77.5
NT	9.1	4.9
94	4.5	9.2
29,52		1.4
84	2.3	1.4
83A,85		0.7
Other	4.5	4.9

Table 3. Phage Types of *Staphylococcus aureus* from Blood Stream of ISR Burn Ward Patients, 1975

Month	Patient No.	No. of Strains	Phage Type					
			84,85	NT	94	84	83A,85	Other
			No. of Strains					
Jan	240 '74 6 7	1 6 1	1 6 1					
Feb	221 '74 237 '74 25 29	3 1 2 2	3 1 1 2	2				
Mar	39 41 47	5 1 1		1				79 -5
Apr	1,8 52 69 79	1 1 1 2	1 1 1		2			
May	*79 63 75 80 83	1 4 1 1 10	4 1 1		1 10			
Jun	89 98 102	4 1 3	2 1 3		2			
Jul	*89 113 138 119	3 2 2 1	3 2 1					42E,47,53,54,75, 83A,81,84 -2
Aug	112 *113 136 144 151 163 165	1 1 1 6 4 3 3	1 1 1 6 4 3 3					
Sep	*151 *153	2 8	2 8					
Oct	*151 189 191 192 196 197 198	2 2 1 6 8 4 4	2 2 1 6 7 4 4			1		
Nov	*192 *196 *198 203 204 216 226	1 2 1 1 2 2 3	2 1 1 2 2 1	2				29,52 -1 29,52 -1
Dec	*216 218 232 239 241	7 1 2 2 1	7 1 2 2 1	2				

Types 94 and 29, 52 were each, recovered from 4.5% of patients. The high proportion of strains of type 94. In contrast to the low percentage of type 29, 52, reflects septicemia with an unusually large number of positive blood cultures from two patients with bacteremia due to type 94.

Type 84 was found in only 2.3% of patients which meant one patient harbored this formerly predominant type. In 1974, 66.6% of blood culture strains were type 84. One patient harbored type 83A, and two others had one strain each of other types.

The chronologic sequence in which staphylococcal sepsis episodes occurred furnished a basis for recognition of circumscribed epidemic outbreaks. Such a sequence is set down in Table 3. The pattern is one of continuous occurrence of episodes of the epidemic type; other bacteremia showed no indication of clustering or of individual outbreaks.

S. aureus from Lung Tissue of Burn Patients at Autopsy

Since pneumonia constitutes one of the principal lethal complications in severe burns, the identity of Staphylococci involved in such episodes was of particular interest. The strains recovered from lung tissue at autopsy were regarded as most significant in their relation to pulmonary involvement. Twenty-six patients, dying with severe burns, yielded 69 strains of *S. aureus* from lung tissue. The type distribution observed is summarized in Table 4. Seventeen, or 65.4% of the patients had type 84, 85 present in the lung. Non-typable strains were recovered from 26.9% of the patients' lung samples. Type 84, type 85, and type 94 were recovered from 4, 2, and one patient respectively. The distribution, in consideration of the small sample size, is to be regarded as consistent with that seen in the blood stream isolates. There was no basis for assuming that a distinctive lung infecting strain had been part of the infection.

Table 4. Phage Types of *Staphylococcus aureus* Strains from Lung Tissues of ISR Burn Ward Patients, 1975

Phage Type	Patients	Strains
	Per Cent	
84, 85	65.4	58.0
NT	26.9	20.3
84	15.4	13.0
85	7.7	4.3
94	3.8	1.4
Other	7.7	2.9

A comparison of the incidence of the principal staphylococcus types in blood, lung and other sites was of importance in its bearing on the relation of specific epidemic type to invasiveness or virulence. Figure 2 summarizes this information. The percent of patients harboring each type in the three categories is represented by the clear outline. The respective sites are differentiated to show the percentage of strains. It was obvious that the specific type 84,85 was most frequently encountered in blood; in contrast, septicemic strains were least frequently found among non-typable strains. Type 94 was most common among other sources, while type 84 was most frequently found in lung samples. Type 29 was found neither in blood nor lung, while type 85 was found only in lung tissue and other sources. The samples suggest that a tissue predilection differing with phage type may exist.

Chronologic sequence of S. aureus Phage Types, 1975

The existence of epidemic strains as represented by annual totals does not convey valid information regarding progression of this process. To elucidate the progress of these staphylococcal episodes, the phage typing data for all strains charted by months, to exhibit patterns of appearance and disappearance of various types Figure 3 summarizes this information. The predominant type was 84,85, in every month. Non-typable strains were second in frequency in each month with one episode, in June, when type 94 was equally common. Type 94 exhibited a capacity for establishing limited increase in frequency, in four additional months. In two months, outbreaks of type 84 were concentrated. The remaining types were essentially scattered, but mainly in Jan-Mar and in Aug-Oct 1975.

Antibiotic Sensitivity of S. aureus Strains, 1970-1975

The epidemic pattern of S. aureus in the ISR burn ward makes obvious the need for effective control of these infections. Sensitivity to antibiotic has varied widely in this Institute over extended periods of time. Methicillin resistance and associated decreased sensitivity to gentamicin and cephalothin was more extensive than has been reported elsewhere. The changes that have occurred since 1968 are summarized in Table 5. In 1968, a high level of sensitivity to methicillin and its analogues was present in the staphylococcal population. In 1969, a drop in proportion of isolates sensitive to methicillin began; it reached its lowest point in 1972, when only 13% of staphylococci were sensitive to methicillin. Oxacillin and nafcillin, although somewhat more effective, also became ineffective. Gentamicin and cephalothin also became less effective. The pattern changed to reappearance of sensitive strains in 1973, and 1974. In 1975, methicillin resistance reappeared, although as yet oxacillin and nafcillin have not lost effectiveness, nor has cephalothin. Minocin resistance increased markedly in 1975, and gentamicin also dropped markedly in in vitro activity against staphylococci. Relatively new antibiotics have been added to the test battery, and among these, Vancomycin has been extremely active against strains thus far tested. Clindamycin, first tested in 1973, displayed a limited degree of effectiveness, but in 1974-1975, almost all strains tested were inhibited. Tests with Vibramycin were in progress in late 1975; the results were promising for in vitro effectiveness.

Figure 2 . Incidence of Predominant Staphylococcus aureus Phage Types from Blood, Lung Tissues, and Other Sources

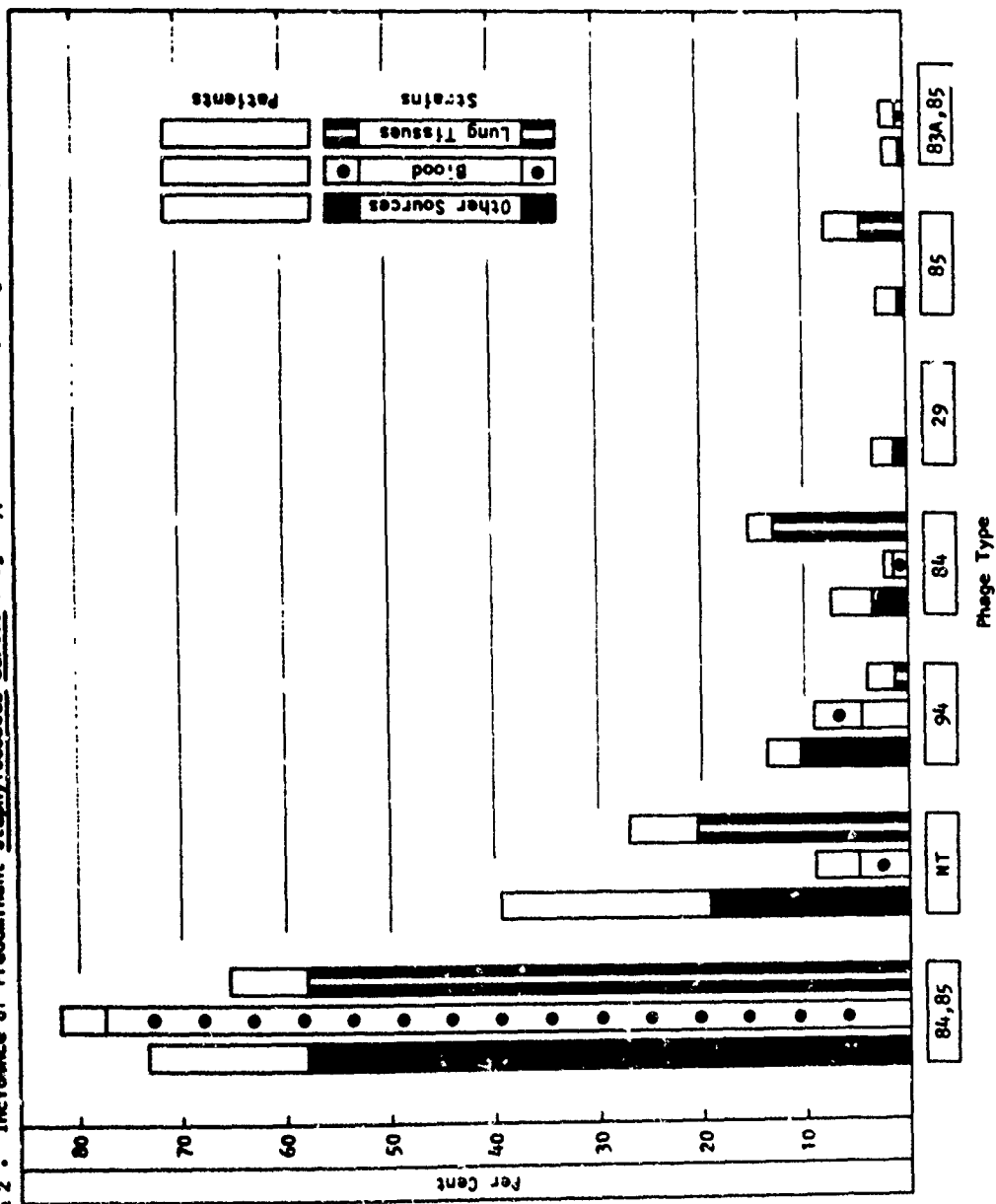


Figure 3. Monthly Distribution of Predominant Staphylococcus aureus Phage Types in ISR Burn Ward Patients, 1975

Phage Type	Month												Total Patients-Strains Each Type
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
84, 85	13-34	10-29	6-27	23-76	19-56	11-27	11-25	18-65	10-31	13-72	12-34	13-41	112-517
NT	4-11	5-15	5-17	15-29	8-17	5-6	4-4	2-2	5-5	3-5	6-10	8-22	60-143
94	3-16	1-1	2-5	4-10	3-34	5-8	2-2	1-1	2-2			1-1	20-80
84	1-1		3-12	7-15		1-2			1-1			1-1	13-32
29	1-2	1-2						1-2	1-1	1-1			5-8
85	1-1	1-3	1-1					1-1		1-1			5-7
83A, 85	1-1		1-1					1-1		1-1			4-4
Total Patients-Strains Each Month	17-71	15-51	14-78	31-134	23-110	16-45	15-34	21-77	16-41	18-86	16-50	18-57	

Table 5. Antibiotic Sensitivity of Staph aureus: % of Strains Inhibited by 6.25 ug/ml or less

Antibiotic	Year							
	1968	1969	1970	1971	1972	1973	1974	1975
Gentamycin	-	52.0	32.0	56.0	35.6	67.9	92.2	38.8
Minocin	-	-	-	-	51.5	84.1	96.0	46.5
Methicillin	84.6	25.7	18.0	15.5	13.1	50.0	82.6	21.8
Oxacillin	80.9	33.0	22.4	20.1	18.8	69.7	65.2	73.6
Nafcillin	90.0	41.0	33.9	33.0	26.0	62.3	83.3	85.6
Cephalothin	-	-	-	56.4	22.6	72.1	90.4	97.2
Vancomycin	-	-	-	-	-	-	-	100.0
Clindamycin	-	-	-	-	-	40.7	95.8	98.0
Vibromycin	-	-	-	-	-	-	-	78.0

Cumulative sensitivity of Staphylococci to antibiotics illustrates with greater precision the nature of sensitivity to antibiotics. Figure 4 shows the sensitivity of Staphylococci to methicillin, oxacillin, nafcillin and cephalothin in 1975. It was evident that cephalothin was by far the most active antibiotic in this group; methicillin was minimally effective, while oxacillin and nafcillin were between these two extremes of activity. The 6.25 mcg/ml level denotes the upper limit of inhibition of strains to be regarded as sensitive. Figure 5 presents the graphic expression of sensitivity of vancomycin, clindamycin, minocin and gentamycin. Action of both minocin and gentamycin, the tetracycline and the aminoglycoside, was minimal; only 45% of strains fell below the 6.25 mcg/ml level. Clindamycin and vancomycin were the most active antibiotics available for control of staphylococcal sepsis. Of these two, vancomycin has been the drug of choice in the treatment of systemic staphylococcal disease, although it was slightly less effective in vitro than was clindamycin.

DISCUSSION

The staphylococcal population in the ISR burn ward has exhibited a unique and unprecedented lability with reference to methicillin. From a typically sensitive population, an almost completely resistant population emerged by progressive change over a three year period. Reversal of this pattern occurred abruptly in 1973; but again, resistance has moved upward in 1975. The degree of lability implied in these shifts has not been previously described. Cross-resistance with tetracycline, aminoglycoside and cephalothins was evident during these shifts. Response to antibiotics active in vitro continues to be a variable entity; this variation points up the need for more effective guidance to antibiotic therapy.

PUBLICATIONS

Lindberg RB, Latta RL, Pruitt BA, Jr: Methicillin-resistant staphylococci in epidemics in a burn unit: Bacteriologic aspect. 15th Interscience Conference

CUMULATIVE SENSITIVITY OF STAPHYLOCOCCUS AUREUS TO ANTIBIOTIC, 1975

Methicillin, Oxacillin, Nafcillin, Cephalothin

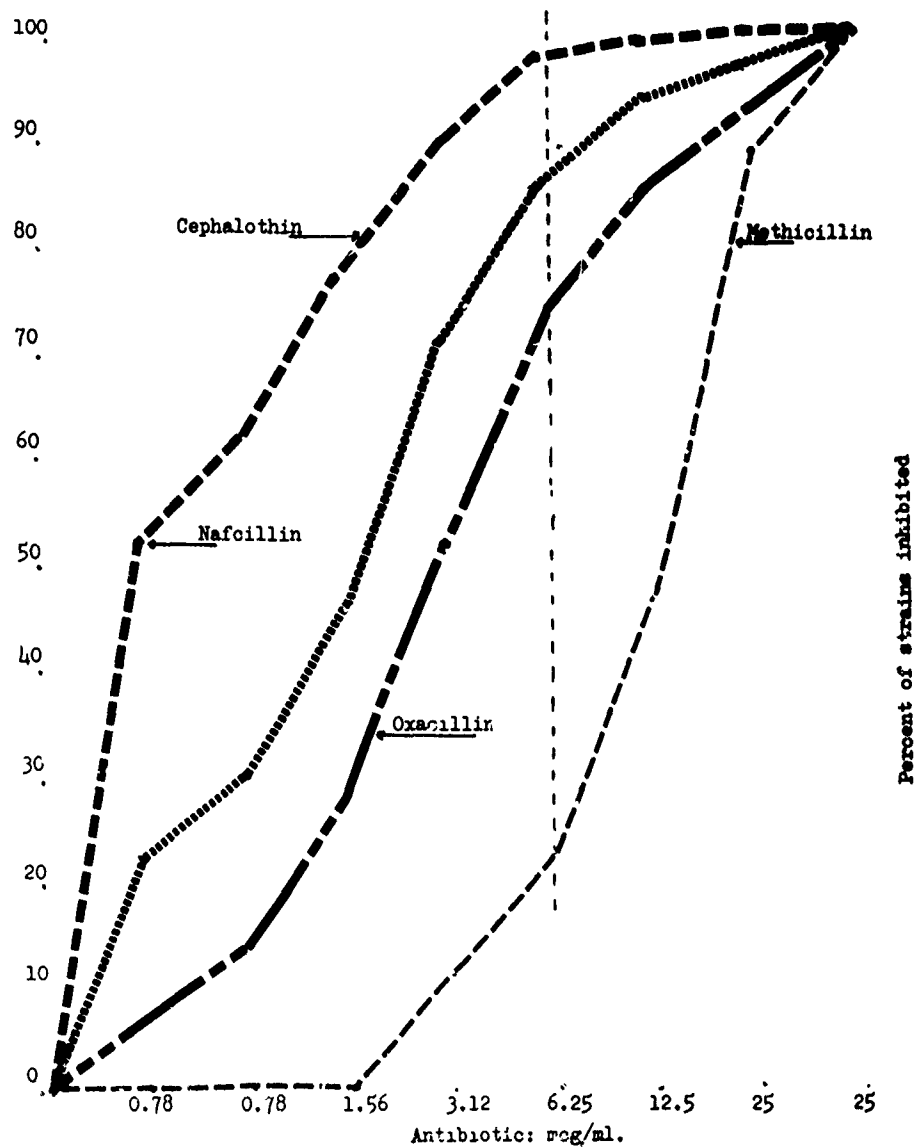


Figure 4.

CUMULATIVE SENSITIVITY OF STAPHYLOCOCCUS AUREUS TO ANTIBIOTIC, 1975

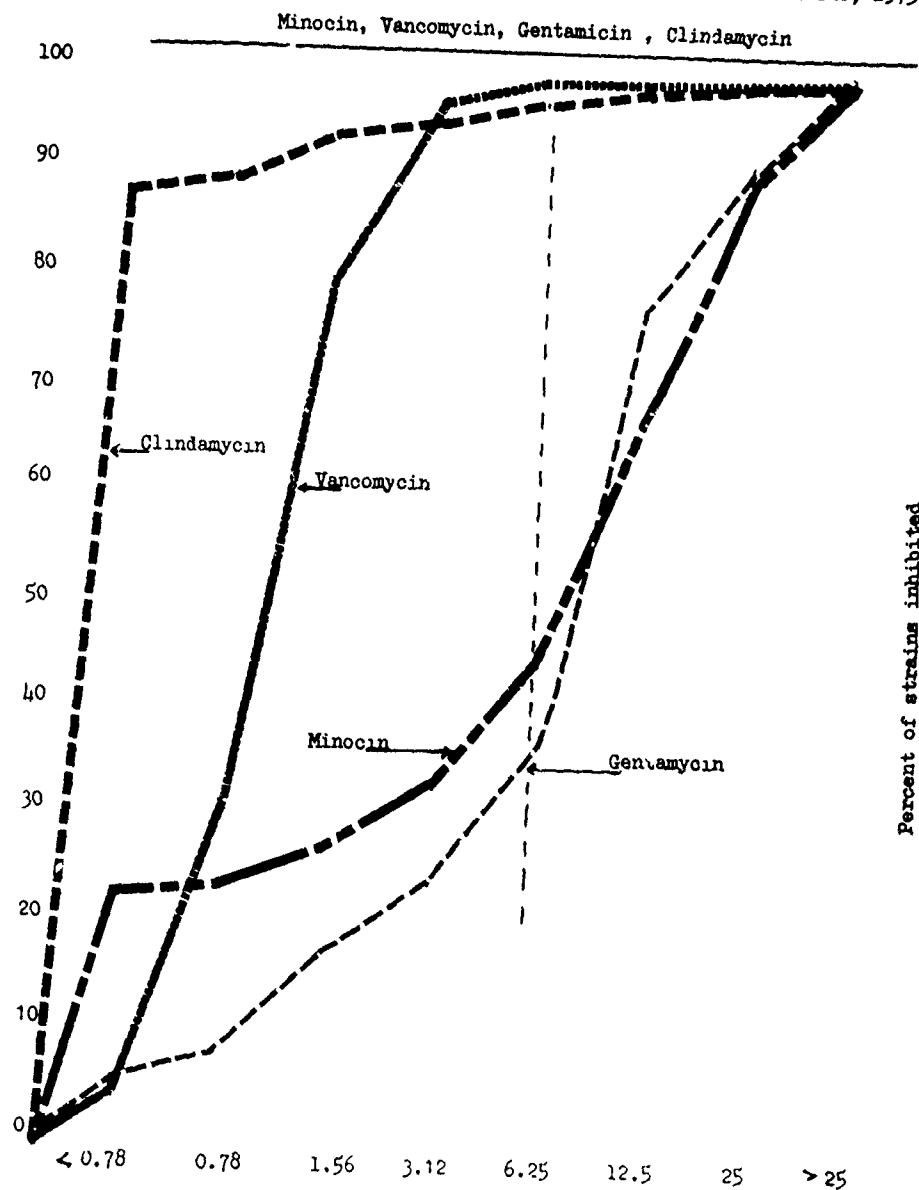


Figure 5.

on Antimicrobial Agents and Chemotherapy. 1975, Abstract 151.

Siegar BE, Long JM, Lindberg RB, Pruitt BA, Jr: Methicillin resistant staphylococci in thermally injured patients: Clinical aspects. 15th Interscience Conference on Antimicrobial Agents and Chermotherpay. 1975, Abstract 152.

PRESENTATIONS

Lindberg RB, Latta RL, Mason AD, Jr, Pruitt BA, Jr: Bacteriologic and Clinical Aspects of Methicillin - Resistant Staphylococcal Epidemics in a Burn Unit Since 1968 at the American Burn Association, San Antonio, Texas, March 31 - April 1, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DMBN INSTR ^a	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61102A	3A161102B71R	01	267			
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Sensitivity To Sulfamylon of Pseudomonas Aeruginosa Recovered From Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
68 07		76 09		DA		C. In-House	
17. CONTR CT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER ^a				76		.5	
c. TYPE:				7T		18	
d. KIND OF AWARD:				FISCAL		.2	
e. AMOUNT:				YEAR		6	
f. CUM. AMT.				CURRENT			
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research			
ADDRESS ^a Fort Sam Houston, Texas 78234				ADDRESS ^a Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U S Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME ^a Robert B. Lindberg, PhD			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2018			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Virginia C. English, MA			
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Pseudomonas; (U) Burns; (U) Sulfamylon; (U) Topical therapy; (U) Humans							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
<p>23. (U) Extensively burned soldiers have been increasingly colonized with <u>Pseudomonas aeruginosa</u> during the past 18 months when initial treatment of burns has been primarily topical silver sulfadiazene. 5% sulfamylon soaks are used to prevent invasion of separating eschar, and hence, the environment offers continuous exposure to this drug. Sensitivity or resistance of opportunists to this agent merits detailed study, since control of infection is a primary objective of combat casualty care.</p> <p>24. (U) Sensitivity is assessed as MIC, in an agar dilution plate. Values are evaluated with epidemic phage types.</p> <p>25. (U) 75 07 - 76 09 In 1975, Sulfamylon sensitivity for <u>Pseudomonas aeruginosa</u> increased to correspond to the median range, with a small but significant resistant population which required 1.25% or greater for inhibition. This confirms the hypothesis of the cluster of resistant strains developing in a transient manner in topically treated wounds.</p>							

^aAvailable to contractors upon originator's approval

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: SENSITIVITY TO SULFAMYLDON OF PSEUDOMONAS AERUGINOSA
RECOVERED FROM BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

Robert B. Lindberg, PhD
Virginia C. English, MS
Basil A. Pruitt, Jr, MD, Colonel, MC
Arthur D. Mason, Jr, MD

Report Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: SENSITIVITY TO SULFAMYLDON OF PSEUDOMONAS AERUGINOSA
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US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
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Report Control Symbol MEDDH-288 (R1)

Six hundred thirty-seven strains of Pseudomonas aeruginosa were monitored for sensitivity to Sulfamylon, using an agar plate MIC dilution method. The strains were more sensitive than the 1972 collection, and even the 1973 set. The median sensitivity level was 0.125% over a 9-year period this value had fluctuated between 0.008 and 0.316%, but the median for 3548 strains was 0.129%. Sensitivity was correlated with predominant phage types; NT-16 and H-1 were sensitive at 0.625%, but the predominant type, E-2, was primarily sensitive to 0.039%. Sulfamylon may be unique in permitting prolonged use with no concrete proof that resistant variants are selected by it.

Pseudomonas
Burns
Sulfamylon
Topical therapy
Humans

SENSITIVITY TO SULFAMYLON OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS

Understanding of the behavior of microorganisms in response to long-term exposure to an antibacterial agent is of basic importance in the control of nosocomial infections. Control of infection in burns involves primarily topical therapy; present practice in the Institute of Surgical Research involves use of both Sulfamylon, burn cream and silver-sulfadiazine cream as topical agents, with follow-up soaks of 5% Sulfamylon used to control invasive infection during the period when eschar is separating and intermittent debridement is carried out to ready the burn site for skin grafting. On the basis of experience with long-term use of other antibacterial agents, it would be expected that bacterial strains resistant to Sulfamylon might be derived by continued exposure of a heterogeneous population to this drug, and continued monitoring of Pseudomonas aeruginosa strains recovered from treated wounds has been carried out since the introduction of Sulfamylon as a generally used medication. The topical agents control other bacteria as well, but Ps. aeruginosa is probably the greatest threat to the burn wound because of its specific proclivity for setting up invasive infection and wound sepsis. This scrutiny of Ps. aeruginosa from a bacterial population exposed to a broad spectrum antibacterial such as Sulfamylon is also merited due to the insight it gives on mechanisms of development of resistance in general, and equally important, a view of the absence of derived resistance among strains in which it does not appear.

Technics of testing sensitivity to Sulfamylon have been described (1). The test is essentially seeding of agar plates containing serial dilutions of Sulfamylon, and reading presence or absence of growth as evidence of inhibition.

SENSITIVITY OF PSEUDOMONAS AERUGINOSA TO SULFAMYLON

There were 637 strains of Ps. aeruginosa tested in 1975. This was the largest number collected in one year; over the past 8 years, the average number tested was 363. Silver-sulfadiazine was first used extensively beginning in 1974 in the Institute of Surgical Research; since its use, the rate of colonization of burns by Ps. aeruginosa has increased to approximately twice what it had been when Sulfamylon was used exclusively.

The sensitivity of the strains in 1975 are set down in Table 1. For comparison, results of tests for the preceding 4 years are presented. The proportion of strains inhibited at levels up to 0.312% was not markedly changed from results recorded in 1974. For the previous 5 years, the sensitivity level has not changed markedly, although an increase in strains requiring 0.625% for inhibition occurred during one year, 1972.

1. Lindberg RB, Calvert J, Brame RE, Dent R: Sensitivity of burn wound flora to Sulfamylon. USA Surg Res Ann Rpt FY 1965, BAMC, Ft Sam Houston, Tx. Section 15.

Table 1
Inhibiting Concentrations of Sulfamylon for Pseudomonas aeruginosa. 1971-1975

Year	Concentration of Sulfamylon in % and Number inhibited									
	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019	< 0.019	
1971	0	0	48	41	56	57	65	13	0	
of total (280)	0	0	17.1	14.6	20.0	20.4	23.2	4.7	0	
1972	0	29	212	46	88	31	37	15	5	
% of total (463)	0	6.3	45.8	9.9	19.1	6.7	7.9	3.2	1.1	
1973	0	4	14	85	85	52	32	12	1	
% of total (285)	0	1.4	4.9	29.8	29.8	18.3	11.2	4.2	0.4	
1974	0	5	59	78	97	97	86	11	4	
% of total (437)	0	1.1	13.5	18.0	22.2	22.2	19.7	2.5	0.9	
1975	1	13	113	108	155	68	147	28	4	
% of total (637)	0.16	2.0	17.7	16.9	24.3	10.7	23.1	4.5	0.64	
Total: 2102	1	51	446	358	481	305	367	79	14	
% of total	0.4	2.4	21.2	17.0	22.9	14.5	17.5	3.8	0.66	

Cumulative sensitivity to Sulfamylon offered a clearer picture of the behavior of Ps. aeruginosa toward this drug. The information is presented in Table 2, for years from 1967 through 1975. Inhibition was complete at 0.625% and almost total at 0.312% from 1968 through 1970. From 1971, onward a slight increase in resistance to Sulfamylon has been manifest. There was a sharp rise in 1972, when over half the strains tested required 0.625% for inhibition, but this figure subsided to a level of between 80 and 85% inhibited by 0.312% from 1973 through 1975. The sensitivity has remained relatively constant since 1972.

Median sensitivity, the level at which 50% of strains are inhibited, offered another measure of the behavior of Ps. aeruginosa in relation to the long-term exposure. These figures are presented for 1967 through 1975 in Table 3. After continuous exposure to the drug for over 10 years, the *Pseudomonas* population was still, and for the past 3 years, has been remarkably consistent in its response to this drug. It is a cliché to speak of acquired resistance on prolonged exposure as being virtually a law of nature, but the experience with Sulfamylon is an exception to this common experience.

Graphic representation of cumulative sensitivity levels since 1971 makes more evident the consistency of behavior of Ps. aeruginosa toward Sulfamylon (Fig. 1). Out of the years shown, in 3 the sensitivity curve was virtually identical. The marked shift in sensitivity in 1972 was returning to this norm during 1973, to account for the right shift in sensitivity during that year.

Throughout studies of the role of Ps. aeruginosa as a pathogen in burns, the importance of recognizing individual strains in relation to a given attribute has been demonstrated. Phage type identity of strains of *Pseudomonas* was correlated with sensitivity. It was shown that certain types, present in micro-epidemic populations of bacteria, were consistent in sensitivity.

Sensitivity to Sulfamylon was not uniformly distributed over the bacterial population. Some strains were quite specific and consistent in sensitivity to Sulfamylon. Other types were less consistent in their behavior with this drug. The results with 4 major strains which typed only with undiluted phage (the NT-strains) are shown in Figure 2. NT-16 and NT-16V (a variant pattern) showed a relatively wide range of sensitivity, as did NT-19V. NT-19 and NT-22, however, showed a preponderance of strains inhibited at one particular level, 0.312% for NT-19 and 0.156% for NT-22.

Strains reacting with Routine Test Dilution in phage typing behaved in a manner comparable to the NT-types. Table 4 shows the behavior of 2 predominant phage types. H-1 included 35 strains inhibited at the relatively high level of 0.625%. However, there were also 12 strains of H-1 more sensitive to Sulfamylon. The attribute is strain-related but not absolute. The E-2V strain was mainly more sensitive, with 15 strains out of 19 inhibited by 0.039% or less.

Phage type E-2 (type 24 in phage number) was highly sensitive to

Table 2. Cumulative Sensitivity to Sulfamylon of Pseudomonas aeruginosa
1967-1975

Year	No. of Strains	Per Cent Concentration and of Strains Inhibited										
		2.50	1.25	0.625	0.312	0.156	0.078	0.038	0.019	< 0.019		
1967	471	100	100	96.8	87.6	81.7	61.3	46.4	15.6	-		
1968	294	100	100	100	95.1	60.4	45.8	14.1	1.7	-		
1969	385	100	100	100	96.5	50.0	26.9	7.7	0.5	-		
1970	296	100	100	100	100	78.0	49.9	21.9	2.0	-		
1971	-	100	100	100	82.9	68.3	48.3	27.9	4.7	-		
1972	463	100	100	93.7	48.0	38.0	19.0	12.3	4.3	1.1	-	
1973	285	100	100	98.1	81.3	57.0	33.5	16.1	3.2	0.4	-	
1974	437	100	100	99.0	85.5	67.5	45.3	23.1	2.4	0.9	-	
1975	637	100	99.8	97.8	80.1	63.2	38.9	24.2	5.1	0.6 ^h	-	

Table 3. Median Value of *Pseudomonas aeruginosa*
Sensitivity to Sulfamylon: 1967 - 1975

Year	No. of Strains	Median Inhibitory Level % Concentration
1967	471	0.083
1968	294	0.136
1969	385	0.176
1970	296	0.068
1971	280	0.125
1972	463	0.316
1973	285	0.111
1974	437	0.086
1975	637	0.125
Total: 9 Years	3548	0.129

SENSITIVITY OF *PS. AERUGINOSA* TO SULFAMYLON 1971-1975

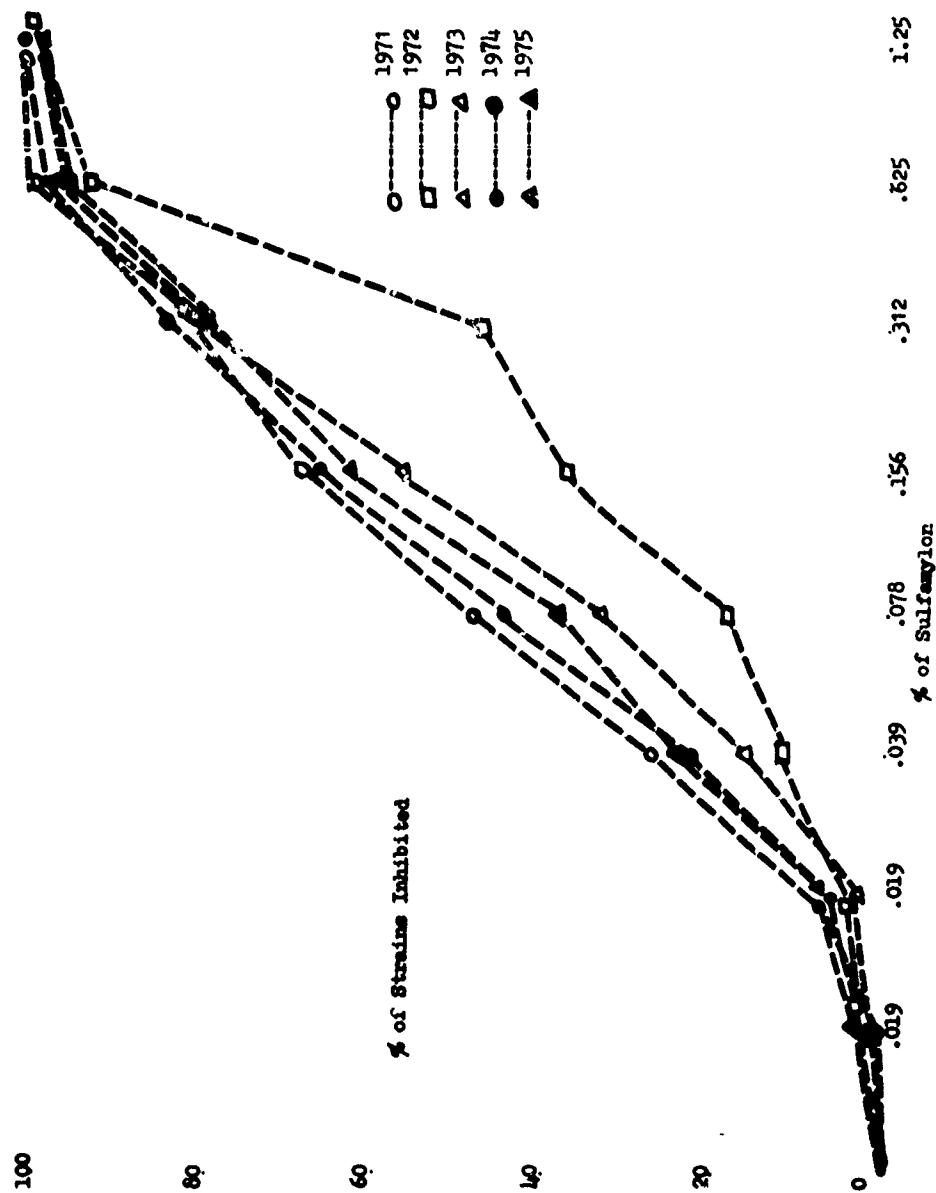


Figure 1.

Sensitivity of Specific Phage Types of *Ps. aeruginosa* to Sulfamylon

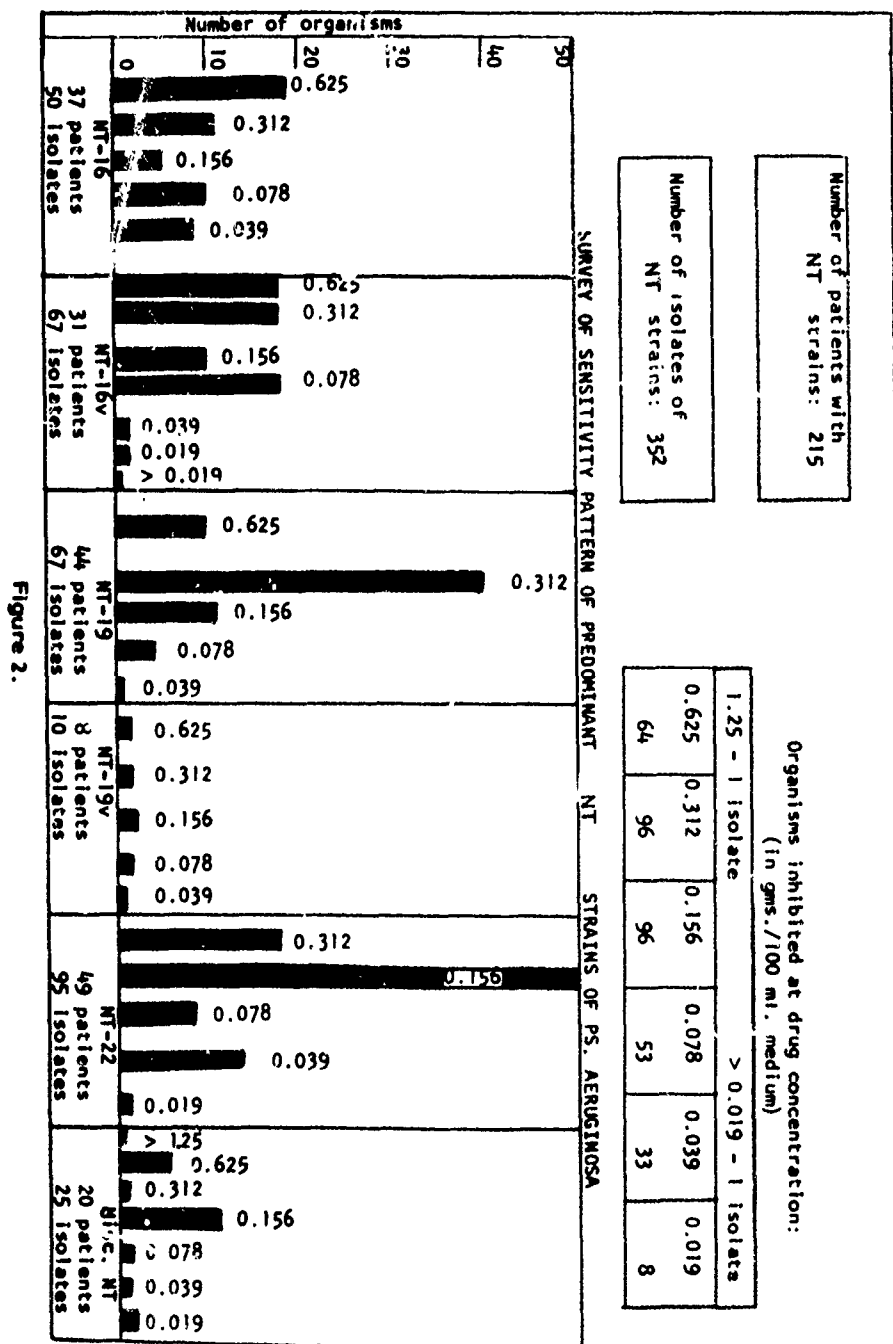


Figure 2.

Table 4.
SULFAMYLON SENSITIVITY REACTION OF VARIOUS
PREDOMINANT PHAGE TYPES - 1975

PATIENT		ISOLATES WITH INHIBITING CONCENTRATIONS* AT							
NO.		0.125	0.625	0.312	0.156	0.078	0.039	0.019	>0.019
Type H-1	93							2	
	160		1						
	159		2						
	166		2						
	191		2						
	152		1						
	144		5						
	163		2						
	26 patients		3						
	151		3						
	126		1						
	199			1					
	161			1					
	181			1					
	150		1						
	139		1						
	47 isolates		2						
	137		3						
	168		1						
	165		1						
	169		1						
	130		1						
	112		1						
	182		1						
	Total each inhibited strain					1		6	
	28							6	
			35	3		1	2	6	
Type E-2 Variant	72					1	1		
	98				1		4		
	63						1		
	102						1		
	12 patients			1					
	55						1		
	75								
	88					1			
	19 isolates						1		
	62						2		
	81								
	90							1	
Total each inhibited strain	127							1	
	119							2	
				1	1	2	11	4	
Type NT-16	12		1						
	33	9	4						
	6		1	1					
3 patients									
20 Isolates									
Total each inhibited strain		12	6	1	1				

*Concentration in gms Sulfamylon/100 ml media

Sulfamylon. Eighty out of 83 strains were inhibited by 0.039% or less; of these 68 were inhibited at that level, as shown in Table 5. The consistency of behavior of an epidemic strain is clearly demonstrated here. Routine testing for antibiotic sensitivity does not segregate individual strains; the data shown here is unusual because it is not often collected. However, the actual pattern of antibiotic sensitivity in nosocomial infections may be equally consistent. The study here reported may well serve as a model for specific strain sensitivity of other species of bacteria, if adequate sorting criteria were collected. This strain-linked sensitivity component prompts investigation of other species for further clues to antibiotic action.

PRESENTATIONS

Lindberg RB. Association of Drug Resistance and Extreme Virulence with Specific Bacteriophage Types of Pseudomonas aeruginosa. Am Assoc. Immunol. Anaheim, CA, April 1976.

PUBLICATIONS

Lindberg RB, Latta RL, Pruitt BA, Jr, Mason AD, Jr: Association of Drug Resistance and Extreme Virulence with Specific Bacteriophage Types of Pseudomonas aeruginosa. Fed Proc 33: 763, 1976 (Abst).

Table 5. SULFAMYLON SENSITIVITY REACTION OF PREDOMINANT
PHAGE TYPE - 24

PATIENT NO. ISOLATES WITH INHIBITING CONCENTRATIONS* AT		0.125	0.625	0.312	0.156	0.078	0.039	0.019	> 0.019
Code - E-2 Type	72						3		
	114								1
	38						2		
	87						2		
	97						1		1
	70						1		
	57						2		
	47						1		
	63						4		
	41						2		
	79					1			
	102						1		
	44						1		
	80						6		
	94								1
	46						1		
	52						1		
	61						1		
	55						1		
	68						1		
	56						7		
	66						2		
	75						1		
	12						1		
	29						1		
	39						1		
	116						1		
	88			1			1		
	62						2		
	90					1		1	
	69						2	2	
	42						1		
	48						1		
	21						2	1	
	27						1		
	113							1	
	54						1		
	36						2		
	60						1		
	73						1		
	53						1		
	51						1		
	74						2		
	103						1		
	85						1		
	40						1		
	25						1		
	112							1	
	109							2	
	119							1	
Total each inhibited strain					1	2	68	9	3

* Concentration in gms. Sulfamylon /100 ml. media

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a		2. DATE OF SUMMARY ^a		REPORT CONTROL SYMBOL	
				DA OB 6950		76 1C 01		DD-DR&E(A)436	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. ORIGIN INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS		9. LEVEL OF SUM	
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		A. WORK UNIT	
10. NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY		61102A		3A161102B71R		01		191	
b. CONTRIBUTING									
c. CONTRIBUTING									
11. TITLE (Precede with Security Classification Code) ^a									
(U) Pathogenesis of Burn Wound Infection: Bacterial Flora of Burn Wounds of Military Personnel Receiving Sulfamylon or Silver Sulfadiazene Treatment (44)									
12. SCIENTIFIC AND TECHNOLOGICAL AREA ^a									
003500 Clinical Medicine									
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY			16. PERFORMANCE METHOD		
65 07		76 09		DA			C. In-House		
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS		20. FUNDS (in thousands)	
Not Applicable				PREEXISTING		.5		15	
a. DATES/EFFECTIVE:				EXPIRATION:		FISCAL		7m	
b. NUMBER: ^a				c. TYPE:		d. AMOUNT:		e. CUM. AMY.	
a. KIND OF AWARD:				b. RESPONSIBLE GOD ORGANIZATION		c. PERFORMING ORGANIZATION		d. PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)	
NAME: ^a US Army Institute of Surgical Research				NAME: ^a US Army Institute of Surgical Research		NAME: ^a Robert B. Lindberg, Ph.D.		TELEPHONE: 512-221-2018	
ADDRESS: ^a Fort Sam Houston, Texas 78234				ADDRESS: ^a Fort Sam Houston, Texas 78234		SOCIAL SECURITY ACCOUNT NUMBER		ASSOCIATE INVESTIGATORS	
RESPONSIBLE INDIVIDUAL				NAME: ^a Basil A. Pruitt, Jr, COL, MC		NAME: ^a Anthony A. Contreras, MS		NAME: ^a Daniel Zamora, SP6	
TELEPHONE: 512-221-2720								DA	
21. GENERAL USE									
FOREIGN INTELLIGENCE NOT CONSIDERED									
22. KEYWORDS (Precede EACH with Security Classification Code)									
(U) Burns; (U) Staph aureus; (U) Enterobacter cloacae; (U) Sepsis; (U) Humans									
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)									
<p>23. (U) The severely burned patient is extremely susceptible to opportunistic bacterial invasion, and topical bacteriostatic therapy has been established as a <u>sine qua non</u> of effective treatment. However, invasive infection with a succession of species of opportunistic normal flora has come to represent the principal cause of death in such injury. Ongoing monitoring is essential to obtain a valid picture of the constantly changing pattern of lethal infection in burns, a major problem in military medicine.</p> <p>24. (U) Wound, sputum, blood, urine, biopsy and catheter cultures, plus quantitative autopsies are used and collated.</p> <p>25. (U) 75 07 - 76 09 Rise in <u>Staphylococcus aureus</u> of a single phage type has been shown. Increase in <u>Pseudomonas aeruginosa</u> and <u>Klebsiella pneumoniae</u> occurred; a 1974 epidemic of <u>Enterobacter cloacae</u> declined, as did all but a remnant of the recent lethal problem species, <u>Providencia stuartii</u>. Findings serve to guide therapy and to aid in developing a more versatile burn wound infection model in animals.</p>									

^a Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 68
AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE -SURGERY

REPORT TITLE: PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL
FLORA OF WOUNDS OF MILITARY PERSONNEL RECEIVING
TREATMENT WITH SULFAMYLON OR SILVER-SULFADIAZINE

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

Robert B. Lindberg, PhD
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Ruth L. Latta, BS
Daniel T. Zamora, SP6

Report Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

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FLORA OF WOUNDS OF MILITARY PERSONNEL RECEIVING
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US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
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Investigators: Robert B. Lindberg, PhD
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Report Control Symbol MEDDH-288(R1)

Bacterial sepsis has continued as the major cause of morbidity and death in burns in 1975. Principal offending species included Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa. K. pneumoniae, although not a newcomer in the area of burn infection, increased to a striking degree as a cause of wound sepsis; it literally replaced Enterobacter cloacae, the previous epidemic species, in early 1975. Ps. aeruginosa was higher in incidence than was the case when topical Sulfamylon[®] was used exclusively in the ISR; its recurrence has accompanied the routine use of silver-sulfadiazine as the primary topical agent now used in the ISR. Self-limited outbreaks, epidemic in pattern, occurred with Serratia marcescens and Escherichia coli; these episodes are reminders that such forms are potential epidemic agents, as far as any control measures for limiting their spread are currently concerned.

Burns
Staphylococcus aureus
Enterobacter cloacae
Sepsis
Humans

PATHOGENESIS OF BURN WOUND INFECTION:
BACTERIAL FLORA OF WOUNDS OF MILITARY PERSONNEL
RECEIVING TREATMENT WITH SULFAMYLON OR SILVER-SULFADIAZINE

The incidence of invasive burn wound sepsis has been strikingly lowered during the past 12 years, by use of effective topical chemotherapy on the burn wound. This reduction was primarily due to control of Pseudomonas aeruginosa. However, since 1969 a continued high level of sepsis in burned patients has prevailed, with a variety of opportunistic pathogens which include Staphylococcus aureus and Enterobacteriaceae spp as the offending organisms. Pseudomonas has not been eliminated, although it seldom causes primary invasive wound sepsis when adequate topical therapy is used. Pseudomonas wound infection, especially in terminally ill burn patients, is common but presently resembles more an opportunistic invasion rather than a specific infectious process. In burn deaths which occur more than 3 weeks post injury, extensive proliferation of gram-negative bacilli occurs in those patients who develop septic shock. This terminal overgrowth of bacteria in the wound usually is made up of several species. Use of appropriate antibiotics has not appeared to improve survival in these conditions.

A previous report ¹ has pointed out that the potentially infected burn surface, modified by such antibacterial agents as Sulfamylon or silver sulfadiazine, harbors a bacterial population which can vary widely in the species present, and in harboring a wide variety of phage types of Ps. aeruginosa. Both topical agents are used extensively, and as with any broad spectrum antimicrobial agent, the wound flora is unquestionably modified as to identity by the presence of these agents. A succession of opportunistic invaders has occurred over the past 7 or 8 years; the conspicuous new epidemic species in 1975 was Klebsiella pneumoniae.

ANTEMORTEM BACTERIOLOGY OF BURN PATIENTS, 1975

The bacterial flora and yeasts recovered from 7 major sites in clinical specimens are shown in Table 1. Two hundred and forty-five patients were admitted in 1975, but 26 of these had no cultures taken. The information shown here represents organisms recovered from cultures from 219 patients. Blood cultures were the most frequent category of specimens and were collected on 92.6% of all patients cultured. This emphasis reflects the concern regarding sepsis as the major problem in microbiology of burns. Out of all specimens submitted 47.8% were blood cultures. The largest number of organisms recovered came from sputum and Luken's tube cultures. Together with wound cultures,

1. Lindberg RB, Contreras AA, Latta RL, Smith HOD, Jr, Zamora DT: Pathogenesis of burn wound infection: Bacterial flora of wounds of military personnel receiving treatment with Sulfamylon or silver-sulfadiazene. USA Inst Surg Res Ann Res Prog Rpt FY 1975, BAMC, Ft Sam Houston, Tx. p. 121.

Table 1. Antemortem Bacteriology of Burn Patients, 1975

Organism	Wound surface	Blood	Source and Number of Isolates				Biopsy	Total Isolates
			Lukens Sputum	Urine	I.V. Cath.	Foley Cath.		
<i>S. aureus</i>	277	174	267	27	57	17	42	861
epidermidis	42	14	71	28	13	5	8	181
Alpha hemol-strep.	24	0	249	6	1	1	0	281
Beta hemol-strep.	4	0	1	3	2	1	0	11
Non hemol-strep.	94	12	163	69	14	24	13	389
<i>Corynebacterium</i> sp.	0	0	2	0	0	0	0	2
<i>Bacillus</i> sp.	15	2	6	1	8	2	1	35
<i>Pseudomonas</i> sp.	383	101	320	120	68	50	83	1125
Mima-Herellea gp.	11	0	19	1	0	0	0	31
<i>K. pneumoniae</i>	209	179	395	164	71	53	46	1117
<i>Enterobacter</i> sp.	2	0	6	0	0	0	0	8
cloacae	107	55	43	58	17	22	11	313
<i>Serratia marcescens</i>	16	29	50	27	10	5	8	145
liquefaciens	0	0	0	1	0	0	0	1
<i>E. coli</i>	72	8	111	48	6	32	17	294
<i>Citrobacter</i> sp.	4	0	5	0	1	1	0	11
<i>Prot. mirabilis</i>	55	8	39	20	6	8	12	148
morganii	2	0	3	0	0	1	0	6
rettgerii	0	0	0	3	0	0	0	3
vulgaris	1	0	0	1	0	0	0	2
<i>Prov. stuartii</i>	24	3	30	10	5	7	2	81
<i>Neisseria</i> sp.	1	0	11	0	0	0	0	12
<i>Candida</i> spp.	55	2	53	75	18	9	7	219
No. patients cultured	176	203	133	157	170	120	63	
No. of specimens	1257	3288	803	738	471	194	135	
Total isolates								5276
Total specimens	6886							
Total patients on whom one or more cultures were done	219							

these cultures offered definitive information as to the source of invasive sepsis when it occurred. S. aureus was the principal gram-positive organism on burn patients. Among gram-negative species, Ps. aeruginosa and K. pneumoniae were recovered in equal numbers, and were by far the most common gram-negative bacilli. Enterobacter cloacae, which had been the predominant species in 1974, became uncommon in June 1975, after an unprecedented predominance in the burn ward for a year and a half.

Chronologic changes in proportion of prominent species are shown in Table 2. Comparison of strain incidence is shown in terms of percent of all isolates recovered in 1975. Ps. aeruginosa increased markedly in incidence; the incidence in the previous 5 years was an average of 12.8%, while in 1975, 21.4% of isolates were Pseudomonas. K. pneumoniae was recovered on a 5 year average, from 11% of clinical specimens prior to 1975; in that year, 21.4% of specimens were positive for Klebsiella. The other species which showed a striking change was Providencia stuartii which was present in only 1.5% of the clinical specimens. Its numbers had decreased steadily since its high point in 1972, when 23.1% of clinical specimens harbored this unusual opportunistic pathogen.

Enter. cloacae was the most recent example of a species of normal intestinal flora which had assumed epidemic proportions as a burn wound invader, in 1974. In early 1975, after a virtual crescendo of cross-infection, it suddenly became relatively uncommon in burn patients.

The number of isolates of each species offers an indication of the relative frequency of occurrence of these opportunistic offenders, but does not indicate how many patients were involved with a given species. The sites cultured and the incidence of patients positive for a given site are summarized in Table 3. Not unexpectedly, staphylococci were most common on the burn itself. Blood, sputum and urine were the sites most often positive for K. pneumoniae. Pseudomonas was most common in biopsies, which are more significant than surface cultures in defining burn wound flora. In contrast to previous years, Enter. cloacae decreased from third to sixth rank in frequency in various specimen sources. Ps. aeruginosa was far more ubiquitous than it had been in the recent past, and K. pneumoniae increased in incidence as an opportunistic invader over the previous 3 years.

BURN WOUND BACTERIOLOGY

The source of bacterial sepsis in a severely burned patient is often obscure. The burn wound itself is frequently described as the entry point for bacteria, and in the specific instance of Pseudomonas burn wound sepsis, the pathognomonic lesion can be demonstrated microscopically.³ However, oppor-

1. Lindberg RB, Mason AD, Jr, Pruitt BA, Jr: Providencia stuartii as a major factor in burn wound infections. Am Soc Microbiol 1973 130 (abstr).

2. Taplitz C, Davis D, Mason AD, Jr, Moncrief JA: Pseudomonas burn wound sepsis. I. Pathogenesis of experimental burn wound sepsis. J Surg Res 4: 200, 1964.

Table 2. Predominant Species Among Isolates from Clinical Specimens
1970-1975

Species	1970	% of All Isolates From Clinical Specimens				
		1971	1972	1973	1974	1975
<i>S. aureus</i>	12.6	15.0	13.8	19.6	18.6	16.4
<i>Pseudomonas</i> sp.	13.6	12.4	13.2	10.4	14.4	21.4
<i>K. pneumoniae</i>	11.5	9.7	11.5	10.1	12.8	21.3
<i>E. coli</i>	6.4	11.0	6.1	10.4	8.2	6.0
<i>Prov. stuartii</i>	21.0	15.2	23.1	15.7	6.6	1.5
<i>Enterocloacae</i>	Not	2.0	3.8	4.3	11.8	6.0
	Differentiated					
% of all isolates	65.1	63.3	67.7	66.2	72.4	66.7
No. of isolates	3293	3179	6696	5672	5689	5254

Table 3. Antemortem Burn Patients Cultured, 1975

Organism	Source and Number of Patients Positive on Culture						
	Wound Surface	Blood	Luken's Sputum	Urine	I.V. Cath.	Foley Cath.	Biopsy
<i>S. aureus</i>	103 (1)	45 (2)	77 (3)	22 (6)	38	15	27 (3)
<i>epidermidis</i>	32	6	38	19	12	15	5
Alpha hemol-strep	21	0	88	5	1	1	0
Beta hemol-strep	3	0	3	3	2	1	0
Gp A strep.	0	0	3	0	0	0	0
Non hemol-strep	46	5	59	42	14	24	13
<i>Corynebacterium</i> sp.	0	0	2	0	0	0	0
<i>Bacillus</i> sp.	9	1	5	1	7	2	1
<i>Pseudomonas</i> sp.	101 (2)	39 (3)	87 (2)	56 (2)	50	33 (2)	41 (1)
<i>Mima-Herellea</i> gp.	9	1	9	1	0	0	0
<i>K. pneumoniae</i>	83 (3)	55 (1)	92 (1)	78 (1)	48	43 (1)	28 (2)
<i>Enterob. aerogenes</i>	2	0	3	0	0	0	0
<i>cloacae</i>	49 (4)	25 (4)	23 (6)	31 (4)	17	22 (3)	9 (5)
<i>Serratia marcescens</i>	13	7	14	16	7	5	4
<i>liquefaciens</i>	0	0	0	1	0	0	0
<i>E. coli</i>	42	9	33	28	6	24	12
<i>Citrobacter</i> sp.	4	0	4	0	1	1	0
<i>Prot. mirabilis</i>	26	4	10	12	5	6	?
<i>morganii</i>	2	0	3	0	0	1	0
<i>rettgerii</i>	0	0	0	2	0	0	0
<i>vulgaris</i>	1	0	0	1	0	0	0
<i>Prov. stuartii</i>	15	2	11	4	5	6	1
<i>Neisseria</i> sp.	1	0	11	0	0	0	0
<i>Candida</i> sp.	31	2	27	29	10	16	6
Total patients							
Samples	176	203	133	157	170	120	63

() relative frequency of patients positive from this site.

tunistic species of Enterobacteriaceae, which so often cause lethal sepsis in severely burned patients, do not exhibit unequivocal sub-dermal invasion nor has it thus far been possible to create a burn wound sepsis model in animals for any bacterial species except Ps. aeruginosa. The wound surface is certainly the most plausible site to exhibit bacterial populations which are altered by topical agents, and observations summarized in this report indicate that this may well have occurred with the change in the Institute of Surgical Research, from Sulfamylon to silver-sulfadiazine as the principal topical agent in early treatment of the burn wound. The environment continued to include the effect of Sulfamylon on the burn, since 5% Sulfamylon soaks have continued in extensive use as a means of controlling bacterial proliferation during the period of eschar separation in the burn. Burn wound surface flora was determined on the basis of cultures on 176 out of the 245 (71.8%) patients admitted. The proportion of patients cultured out of all patients admitted has tended to become less with the increasing awareness that, in the two extremes of minor injury and of massive burns, the identity of the bacterial flora in the burn is of little value as a guide to therapy.

Bacterial species recovered from burn wounds, either from the wound surface or from biopsy, are summarized in Table 4. The highest incidence of colonization occurred with S. aureus and Ps. aeruginosa, with each of these species recovered from over half of the patients cultured. The predominant form of Staphylococcus was type 84,85; however, in terms of overall incidence, there were 25% fewer patients with staphylococcal colonization. Staphylococcus epidermidis appeared in fewer than 20% of patients as had been the case in earlier years. Non-hemolytic streptococci, primarily group D, were also relatively infrequent in occurrence. The incidence of non-hemolytic streptococci is, on an annual basis, relatively consistent.

K. pneumoniae was the third most common species recovered from the burn wound, and was found with twice the frequency observed in wounds in 1974. In this respect it crowded out Entero. cloacae which was recovered from 27.8% of the patients in contrast to the 44.4% found positive in 1974. The other striking change in wound flora was the marked decrease in Prov. stuartii, which appeared on wounds of only 8.5% of patients, from an incidence of 41.6% in 1974. This erstwhile epidemic pathogen became almost a rarity in 1975. Other enteric species, Escherichia coli and Proteus mirabilis, have been recovered from fewer than 1 out of 5 burned patients.

One organism which, although usually rare on burn wounds is continually sought, is the group A beta-hemolytic streptococcus. There is a sound basis for this surveillance; although it can be readily controlled by penicillin, these streptococci can rapidly destroy autograft if they seed the wound. When it appears on the burn ward, in any individual, it must be eradicated by antibiotic prophylaxis, and survey of the population is called for. Table 5 illustrates the wide fluctuation in incidence of group A streptococci that has been observed since 1969. There were two years, 1972 and 1974, in which relatively large numbers of patients were colonized with group A streptococci. In 1975, only 3 strains were recovered. None of these involved an actual wound infection, and each was eradicated promptly by administration of penicillin. However,

Table 4. Burn Wound Surface Flore in 176 Patients
ISR, 1975

Species	No. of Strains Recovered	No. of Patients Positive on Burn Surface	% of Cultured Patients Positive
<i>S. aureus</i>	277	103	58.5
<i>epidermidis</i>	42	32	18.2
Strep, non-hemolytic*	94	46	26.1
<i>K. pneumoniae</i>	209	83	47.2
<i>Enterobacter cloacae</i>	107	49	27.8
<i>E. coli</i>	72	42	23.9
<i>Proteus mirabilis</i>	55	26	14.8
<i>Providencia stuartii</i>	24	15	8.5
<i>Pseudomonas</i> sp.	383	101	57.4

* These strains primarily group D.

the potential emergence of streptococci in a burn population requires continuing monitoring of this species.

Table 5. Incidence of Group A Streptococci on
Burn Wounds

Year	No. of Strains of Group A Streptococci Recovered
1969	8
1970	2
1971	1
1972	56
1973	3
1974	30
1975	3

RESPIRATORY TRACT FLORA IN BURNS

Pneumonia is a major threat in the severely burned patient. The bacterial species associated with pulmonary complications are those that also colonize the burn wound, although the proportional distribution of species may vary from that seen on the burn. There were 803 specimens of sputum cultured from 133 patients in 1975. Thus, 54.2% of patients admitted

had at least one sputum culture. An average of 6 specimens per sampled patient were cultured.

Trends in species occurring in pneumonia in the burned patient are shown in Table 6. Incidence of the major species of significance in the ISR since 1971 are summarized here.

Table 6. Principal Species of Bacteria Recovered from Respiratory Tract of Burned Patients, 1971-1975

Species	% of Patients Exhibiting Positive Sputum on Culture				
	1971	1972	1973	1974	1975
<i>S. aureus</i>	43.0	38.5	56.9	59.2	57.9
<i>K. pneumoniae</i>	45.0	58.8	60.0	68.4	69.2
<i>Enterobacter cloacae</i>	11.0	27.0	23.8	35.7	17.3
<i>E. coli</i>	27.2	40.9	53.8	33.7	24.8
<i>Proteus mirabilis</i>	-	19.0	10.8	21.4	7.5
<i>Providencia stuartii</i>	33.0	56.5	40.8	59.2	8.3
<i>Pseudomonas sp.</i>	39.0	38.5	36.2	63.3	65.4
Patients cultured	94	122	130	98	133

An increase in incidence of *S.aureus* in sputum occurred in 1973, and this trend was continued through 1975. This increase was reflected in a concomittant rise in rate of recovery of staphylococci from the lung of burn fatalities at autopsy.

A more gradual but consistent rise in rate of recovery of *K.pneumoniae* from sputum was evident. This species was the predominant organism in sputum over the entire 5 year period. Out of 72 autopsies in which complete cultures were made, 40 (55.5%) showed *K. pneumoniae* as predominant in at least one lung lobe. Out of the same series, 31 (43%) had one or more lobes predominant for *Ps. aeruginosa* and 20 (27.7%) had *S.aureus* predominant. The high rate of recovery of *Klebsiella* in sputum thus was paralleled by its predominance in the lung tissue at death. Its pathogenic role was more than ever predominant during 1975.

Ps. aeruginosa increased markedly from 36.2% positive in sputum in 1973 to 64% in 1974 and 1975. This change coincided with the period of introduction of silver sulfadiazine as a topical agent in early treatment of burns, and it closely paralleled the recovery rate of *Pseudomonas* from burn wounds. The recovery rate was consistent for 1974 and 1975.

Enterocloacae went from the status of a very prominent species in burn sepsis in 1974 to a low incidence in sputum and in surface coloni-

zation during this period. The incidence in sputum in 1975 was half that found in 1974. Lung tissue at autopsy showed a comparable decrease in this species, which adds weight to the significance of the sputum culture as an index of invasive infection in the lung. Only 3 autopsies out of 72 had Enterocloacae predominant in at least one of 4 lobes of the lungs; one of these was predominant in 3 lobes. In 1974, out of 74 autopsies exhibited a predominance of Enterocloacae in lung tissue. In the 23 patients with positive sputa in 1975, there were only 43 specimens positive. Thus, even when Enterocloacae appeared in sputum it was non-persistent.

SEPTICEMIA AND BACTEREMIA IN BURNED PATIENTS

The continuing lethal problem in severe burns is sepsis, and in consequence the most significant data regarding relative importance of opportunistic invaders is the population recovered in blood cultures. The impression remains strong that the identity of blood stream invaders may be a fact secondary to the existence of an altered susceptibility of the host, so that ultimate control of this problem lies with preventing this collapse of defense capability. However, survival may also be favorably enhanced by control of offending strains of gram-negative opportunistic invaders.

In 1975, 203 out of 275 patients (Table 7) contributed blood cultures during hospitalization. One hundred of these individuals had at least one positive blood culture. There were 12 genera or species differentiated among bacteremic strains; only 4 of these were of major numerical importance. K. pneumoniae was recovered from the highest proportion of patients positive, and over three-fourths of patients with this species in the blood stream expired. Mortality accompanying gram-negative bacteremia has consistently been higher than that seen with staphylococcal sepsis. Fifty-seven per cent of patients with staphylococcemia expired in 1974; the same ratio was observed in 1975. Fatality rates for patients with gram-negative sepsis ranged from 69% to 78% for the 3 numerically important species.

Septicemia in severely burned patients usually involves more than one species of organism in successive cultures. The mortality seen in patients in whom only one species was recovered furnish a more precise insight into the relationship of invading organism to outcome. Only 3 species of organism were recovered as one-species bacteremias. Table 8 summarizes the result in these cases. Survival prospects in patients exhibiting only staphylococcemia were relatively favorable. For *Klebsiella* and *Enterobacter* sepsis, the survival rate was far less. The other 9 species recovered from burn patients were found as part of a polymicrobial septicemia.

The total of blood cultures from which the septicemia data was drawn in 1975 was modified somewhat by a study conducted on the clinical service, in which multiple blood cultures were drawn during manipulations of the wound. Surgical debridement under anesthesia and hydrotherapy treatment were the 2 modalities studied. It has been assumed that septicemia might arise from manipulation of the colonized wound, which would make more probable the entrance of bacteria into the blood stream. These observations were directed

Table 7. Blood Culture Isolates from 203 Burned Patients,
1975: Relation of Species Recovered to Mortality

Species Recovered	No. Patients Positive	No. Isolates	% of all Patients Cultured Positive	No. Patients Who Expired	% of all Patients With Positive Cultures Who Expired
<u>S.coag.Pos.</u>	45	174	22.1	26	57.7
<u>S.coag.Neg.</u>	6	14	3.0	1	16.7
Non-hemol.Strep.	5	12	2.5	5	100.0
<u>Bacillus sp.</u>	2	2	0.9	1	50.0
<u>Ps.aeruginosa</u>	39	101	19.2	27	69.2
<u>K.pneumoniae</u>	55	179	27.0	43	78.2
<u>Enterocloacae</u>	29	55	14.3	20	68.9
<u>Serratia marcescens</u>	7	29	3.4	6	85.7
<u>E.coli</u>	7	9	3.4	3	42.5
<u>Proteus mirabilis</u>	4	8	2.0	3	75.6
<u>Providencia stuartii</u>	2	3	0.9	1	50.0
<u>Candida sp.</u>	2	2	0.9	2	100.00
No. Patients with at least 1 positive blood culture:			100		

Table 8. Bacteremia with Only One Species of
Bacteria Recovered: Burn Patients, 1975

Species	No. Patients With One Species Recovered	Average No. Positive Blood Cultures/ Patient	Deaths	% Mortality For One Species Septicemia
<i>S. aureus</i>	12	3.9	3	25.0
<i>K. pneumoniae</i>	16	3.3	10	62.5
<i>Enterocloacae</i>	3	1.9	3	100.0

toward answering that question.

Twenty-eight patients were sampled with from 3 to 8 successive cultures collected before, during and after manipulation of the burn wound. Of these 16 showed no positive blood cultures at any time during their course, including the manipulation. Twelve patients had one or more positive blood cultures. Of these, 5 patients, whose burns were large, died, with an average burn size of 63.0% of the body surface. The 7 survivors of at least one positive blood culture had body surface burns averaging 38.9%. Table 9 compares the results of intensive sampling before, during and after manipulation of the burn wound, with the blood stream flora indicated by cultures collected on the basis of clinical status, on days when no manipulative procedures were performed. Among the 5 fatal burn cases, the similarity of the 2 sets of conditions was obvious; organisms recovered in the manipulated patient were quite comparable to those recovered on days when no manipulation occurred. Among the 7 cases who survived, 4 had no blood cultures positive under routine clinical conditions, but did yield staphylococci (2 cases), and one patient each with a *Klebsiella* or a *Pseudomonas* strain recovered. Such recoveries occurred in a total of 5 procedures; a total of 18 series of cultures were negative under similar conditions. Three patients displayed bacteremia with staphylococci (2 patients), *Klebsiella* (2 patients) and 1 *Serratia* positive. Under manipulation, the staphylococcus appeared in 2 patients, while 1 transient *Klebsiella* bacteremia yielded a *Pseudomonas* after manipulation. The contribution of this special set of observations to the total picture of septicemia in severely burned patients was not large enough to justify exclusion of these patients from the total for 1975, and indeed, the implication that such manipulation markedly affected bacteremia was not strongly supported. A slight increase in variety of species could be discerned in the septicemic patients, when they were subjected to manipulation, but no persistent conversion from sterile to contaminated blood stream occurred.

Blood cultures in 62 patients, or 62% of those with bacteremia, were positive for more than one species in successive cultures. In some instances

Table 9. Patients with Positive Blood Cultures During Wound Manipulation

Outcome & Patient No.	% Burn	Cultures Drawn Other Than On Day of Manipulation	Cultures Drawn During Manipulation	
<u>Died</u>		<u>Organism Recovered</u>	<u>Organism Recovered</u>	<u>No. of Culture Sets Drawn</u>
1	57.5	Klebsiella; Staph, Pseudo.	Klebsiella; Pseudo; Serratia; Proteus	2
2	80.5	None collected	Klebsiella; Staph.	1
3	89.0	Staph; Klebsiella; Pseudo; Enterobacter; Serratia	Serratia; Proteus; Pseudo; E. coli	2
4	47.0	Staph; Klebsiella	Staph; Pseudo; Klebsiella No growth	6 1
5	71.0	Klebsiella	Klebsiella No growth	2 1
<hr/>				
<u>Survived</u>				
6	54.5	No growth	Staph. No growth	1 6
7	63.0	No growth	Staph. No growth	2 7
8	38.0	No growth	Klebsiella no growth	1 1
9	44.5	Staph; Klebsiella	Staph; Klebsiella No growth	4 2
10	39.5	Staph; Serratia Enterobacter	Staph; Klebsiella	1
11	21.0	Klebsiella	Pseudo. No growth	1 3
12	12.0	No growth	Pseudo. No growth	1 4

a species would appear, disappear, then reappear, and in fatally ill patients a terminal period of mixed blood cultures was not uncommon. The extreme heterogeneity of species observed in blood cultures is shown in Table 10. As has been pointed out in previous summaries, the absence of a consistent pattern of mixed cultures is a conspicuous feature of sepsis in the severely burned patient. Staphylococci were associated with 19 different combinations of Enterobacteriaceae species; the most common pairing of S. aureus with Enterocloacae or with Ps. aeruginosa occurred in only 4 of each out of 31 patients. The most common pair was Klebsiella and Pseudomonas, in 8 patients out of 19 with Klebsiella bacteremia. Recurrent proposals to immunize burned patients to protect them against bacterial sepsis have been submitted. The bacterial flora of the blood stream in these severely burned patients suggests that this desirable end may indeed be a fond hope; while mobilization of versatile defense mechanism against this plethora of opportunistic invaders is not inconceivable, it does not appear plausible. It is important that the ubiquitous role of staphylococci in this redundant accumulation of species not be overlooked in any regimen designed to control sepsis.

BIOPSIES AND BURN WOUND FLORA

Biopsy as a diagnostic procedure has become well established technic. It is used primarily to assess the status of potential invasive wound infection, and also to evaluate the degree of success that therapy has attained. The bacterial population found in sub-surface tissue of the burn area is not of necessity a reflection of the surface colonizing flora. The predominant organism in a biopsy sample of burn wound may give a clue as to the identity of the most significant part of the burn wound flora, as far as potential invasive infection is concerned.

Table 11 presents the bacteriologic results collected on biopsies on 65 patients during 1975. This was 25.7% of all patients admitted in 1975, and contrasts to the 71.8% of admissions on whom wound surface cultures were collected. The numerically important species were Ps. aeruginosa, K. pneumoniae, and S. aureus. Non-hemolytic streptococci, E. coli, and Enterocloacae were present in biopsies on from 21% to 14% of the patients. When the survival rate in relation to species recovered from biopsy was assessed, the highest mortality rates were recorded for Klebsiella, E. coli, and non-hemolytic streptococci. Fatal outcome from Pseudomonas and Enterocloacae were virtually identical in recovery at 55.6% to 56.1% of patients. S. aureus was associated with a lethal sepsis in but 44.4% of patients.

During the past decade it has been observed that major burn wound infecting organisms vary in identity over a long period of time. Illustrative of this hypothesis are the sequences of Prov. stuartii, Enterocloacae and K. pneumoniae that have successively dominated the nosocomial infection pattern in the Institute of Surgical Research since 1969. Table 12 summarizes the annual incidence of the major species in biopsy samples and also displays the mortality rate associated with each species. S. aureus has consistently been recovered in from 40% to 50% of patients biopsied. The behavior of this species, even when a succession of phage types has been present,

Table 10. Blood Culture Isolates in Patients with
More than One Species Recovered

<u>Species</u>		<u>No. of Patients</u>
S. aureus, Pseudomonas sp.		4
S. aureus, Entero. cloacae		4
S. aureus, K. pneumoniae		3
S. aureus, E. coli		1
S. aureus, K. pneumoniae, E. coli		2
S. aureus, K. pneumoniae, Entero. cloacae		2
S. aureus, Entero. cloacae, Pseudomonas sp.		2
S. aureus, S. epidermidis, Pseudomonas sp.		1
S. aureus, Pseudomonas sp., Non-hemolytic strep.		1
S. aureus, K. pneumoniae, Pseudomonas sp.		1
S. aureus, K. pneumoniae, Pseudomonas sp., S. marcescens		1
S. aureus, K. pneumoniae, Pseudomonas sp., Entero. cloacae		3
S. aureus, K. pneumoniae, Pseudomonas sp., Prov. stuartii		1
S. aureus, K. pneumoniae, Pseudomonas sp., Mima-Herellea gp.		1
S. aureus, K. pneumoniae, E. coli, Prot. mirabilis		1
S. aureus, K. pneumoniae, Entero. cloacae, S. marcescens		1
S. aureus, K. pneumoniae, Pseudomonas sp., S. marcescens, Prot. mirabilis		1
S. aureus, K. pneumoniae, Pseudomonas sp., Entero. cloacae, non-hemolytic strep		1
S. aureus, K. pneumoniae, Pseudomonas sp., S. marcescens, Prot. mirabilis, E. coli		1
K. pneumoniae, Pseudomonas sp.		8
K. pneumoniae, S. marcescens		1
K. pneumoniae, Entero. cloacae		4
K. pneumoniae, S. epidermidis		1
K. pneumoniae, Pseudomonas sp., Candida sp.		1
K. pneumoniae, Pseudomonas sp., Non-hemolytic strep.		1
K. pneumoniae, Pseudomonas sp., Entero. cloacae		1
K. pneumoniae, E. coli, Candida sp., S. marcescens		1
K. pneumoniae, Entero. cloacae, Pseudomonas sp., Non-hemolytic strep.		1
Entero. cloacae, Prov. stuartii		1
Entero. cloacae, Pseudomonas sp.		6
E. coli, Pseudomonas sp.		1
E. coli, S. marcescens		1
Bacillus sp., S. epidermidis, Pseudomonas sp.		1
Non-hemolytic strep., Prot. mirabilis		1
Total patients with 2 or more species		62
		<u>% of All Positives</u>
No. of patients with 2 species	35	35
No. of patients with 3 species	14	14
No. of patients with 4 species	10	4
No. of patients with 5 species	2	2
No. of patients with 6 species	1	1
No. of patients with Entero. cloacae	26	
S. aureus	32	
Prov. stuartii	2	
K. pneumoniae	38	
Pseudomonas sp.	35	

Table 11. Bacterial Flora of Biopsies on Burn Wounds of
63 Patients, 1975

Species	No. Patients Positive	% of Patients Positive	No. Patients with Positive Cultures Who Expired	% of Patients Positive Who Expired
<i>S. aureus</i>	27	49.2	12	44.4
<i>S. epidermidis</i>	5	8.0	1	20.0
Non-hemol. strep	13	21.0	8	61.5
<i>Bacillus</i> sp.	1	1.6	1	100.0
<i>Ps. aeruginosa</i>	41	65.1	23	56.1
<i>K. pneumoniae</i>	28	44.4	22	78.6
<i>Enterocloacae</i>	9	14.3	5	55.6
<i>E. coli</i>	12	19.0	9	75.0
<i>Prot. mirabilis</i>	3	4.7	3	100.00
<i>Prov. stuartii</i>	1	1.6	1	100.00
<i>Candida</i> sp.	6	9.5	3	50.0
<i>S. marcescens</i>	4	6.3	3	75.0

No. of specimens collected: 135
No. of samples per patient (average): 2.1

Table 12. Burn Wound Biopsy Flora: Species Incidence and Mortality
1969 as Compared with 1971-1975

Species	% of Patients Positive					% of Patients Positive Who Expired						
	1969	1971	1972	1973	1974	1975	1969	1971	1972	1973	1974	1975
<i>S. aureus</i>	42	44	41	51.9	50.4	42.9	22	38	22	50.9	45.6	44.4
<i>K. pneumoniae</i>	20	17	32	17.0	14.1	44.4	50	31	19	55.5	78.9	78.6
<i>Enter. cloacae</i>	-	-	-	19.8	47.3	14.3	-	-	-	61.9	59.3	55.6
<i>E. coli</i>	14	19	27	25.5	20.7	19.0	47	33	16	65.6	67.9	75.0
<i>Prov. stuartii</i>	51	40	56	36.8	32.6	1.6	14	58	36	51.0	70.5	100.0
<i>Prot. mirabilis</i>	34	13	14	9.4	9.6	4.7	38	40	9	60.0	53.8	100.0
<i>Ps. aeruginosa</i>	30	30	32	32.1	23.0	65.1	39	57	20	50.0	67.7	56.1

is consistent with the hypothesis that it is an endogenous part of the burn flora. Despite the availability of antibiotics effective against staphylococci, its incidence has remained constant. Although it is ubiquitous, it has not increased in rate of spread over the past 8 years. Prov. stuartii presents the most dramatic example of a species of Enterobacteriaceae which reached a high level of spread in burn wounds, at the same time when it was a major cause of sepsis. It then diminished in tissues biopsied, from 56% of patients in 1972 to 32% of patients in 1974, after which it virtually disappeared from invaded burn wounds. When it does again appear, it is associated with a high mortality rate. Entero. cloacae was of so little consequence in burn patients that it was not differentiated as a species until 1973. In that year it was found in 20% of patients biopsied. In 1974 it was present in almost half of biopsied patients, and then, in 1975, it virtually disappeared. The 14% incidence rate in 1975 is made up of the samples collected in the first half of the year. Its implications with regard to patient survival have remained relatively constant; over half of the patients with Entero. cloacae in wound biopsy expire. K. pneumoniae became the predominant species in 1975; it was found in half the patients biopsied, and on a monthly, not annual basis, it became far more common in the second half of the year. A disturbing rise in mortality rate, among patients with Klebsiella-positive biopsies has occurred; among predominant organisms found in tissues, it was associated with a continuously rising mortality rate, which reached 78.6% of patients thus positive in 1975.

KLEBSIELLA PNEUMONIAE IN BURN PATIENTS

The change from one species to another of opportunistic invasive coliform bacillus in burn ward patients has prompted close study of the offending species. The emergence of K. pneumoniae in 1975 was a classic example of an epidemic of a pathogen which had not previously shown marked invasive potential in burn wounds. The extent of involvement of burn patients with Klebsiella in various sites is shown in Table 13. Autopsy data from burn wound and lung are also presented. This predomance of Klebsiella at autopsy adds weight to the implication that the antemortem Klebsiella bacteremia was clinically significant in the patients' demise.

An association between incidence of Klebsiella sepsis and any increase in seeding of the burn patient with K. pneumoniae was sought. Table 14 summarizes the incidence of Klebsiella seeding associated with septicemia over the 1971-1975 period. Seeding of the wound surface fluctuated over a range of 25% to 47%; sputum cultures similarly were positive in from 45% to 69% of patients. The incidence in 1975 was the highest recorded, but it was high also in 1974. Biopsy cultures, however, were more consistent with the rise in incidence seen in blood cultures. The conservative interpretation of these findings would suggest that, while surface and respiratory tract flora varied, tissue invasion as reflected in biopsy findings more nearly paralleled the rise in incidence of bacteremia. The incidence of positive wound and sputum cultures was sufficiently high to indicate that prompt and consistent transfer of Klebsiella from patient to patient in the burn wards was occurring.

Table 13. *Klebsiella pneumoniae*: Isolates from Clinical and Autopsy Specimens, 1975

Source	No. Isolates/ Total Specimens	% of Specimens Positive	No. Patients Positive/ Total Patients Cultured	% of Cultured Patients Positive
Burn wound, swab, clinical	209/1257	16.6	83/176	47.2
Biopsy, wound	46/135	34.1	28/63	44.4
Blood culture	179/3288	5.4	55/203	27.1
Sputum (Luken's)	395/803	49.2	92/133	69.2
Urine	164/738	22.2	78/157	49.7
Foley catheter tip	53/194	27.3	45/120	37.5
I. V. catheter tip	71/471	15.1	45/170	26.5
Autopsy: burn	136/261	52.1	48/75	64.0
Autopsy: Lung	156/287	54.4	60/75	80.0

Table 14. Per Cent of Patients Cultured Who Harbored *Klebsiella pneumoniae*, 1971-1975

Year	Site and Per Cent Positive			
	Wound Surface	Biopsy	Blood	Sputum
1971	25.2	17.0	5.0	45.0
1972	43.8	32.0	9.5	58.8
1973	33.3	17.0	6.6	60.0
1974	25.7	14.1	14.4	68.4
1975	47.2	44.4	27.0	69.2

The epidemiologic shift from *Enterobacter cloacae* to *K. pneumoniae* has been demonstrated as the major event in septic infection in burns in 1975. As a corollary, the change in incidence of *Enterobacter cloacae* in tissues and fluids of burn patients has been summarized to complete this sequence, which was not only intrinsically dramatic and unexpected, but also the most well documented episode thus far observed, in which 2 closely related species acted in succession to set up epidemics in a susceptible patient population. This occurred abruptly and resulted in a new nosocomial infection of great lethality. Table 15 shows the reciprocal level of *Enterobacter cloacae* and *K. pneumoniae* in various sites in 1974 and 1975. The replacement *Enterobacter* by *Klebsiella* was especially notable in biopsy, blood and post mortem tissues. Among the latter, the reciprocal replacement of *Enterobacter* by *Klebsiella* was numerically very precise.

Table 15. *Klebsiella pneumoniae* and *Enterobacter cloacae*: Isolates from Clinical and Autopsy Specimens 1974-1975

Site	Per Cent of Cultured Patients Positive			
	<u>Enterobacter</u> <u>1974</u>	<u>cloacae</u> <u>1975</u>	<u>Klebsiella</u> <u>1974</u>	<u>pneumoniae</u> <u>1975</u>
Wound	35	27.8	25.7	47.2
Biopsy	47.3	14.3	14.1	44.4
Blood	20.2	14.3	14.1	27.1
Sputum	35.2	17.3	68.4	69.2
Urine	32.6	19.7	27.9	49.7
I.V. Tip	20.5	10.0	13.1	26.5
PM* Lung	30.6	10.7	9.5	36.5
PM Liver	27.5	2.0	10.8	80.0
PM Wound	40.0	32.0	36.0	64.0

* PM: post mortem

CATHETER TIP CULTURES

The etiology of intravascular infection in severely burned patients is in most instances associated with the indwelling catheter as a source of infection, either as an avenue for ingress of infecting organisms or as a nidus on which circulating bacteria may attach. I.V. catheter tips are routinely cultured as part of the ISR operation at time of removal from the patient. Table 16 summarizes the observations on organisms recovered from this source. Four hundred seventy-one catheter tips from 170 burn patients were cultured. Ninety-one, or 53% , of these patients had positive cultures for at least one I.V. tip. Numerically important species, in order of frequency of occurrence, were Ps. aeruginosa in 55% of patients, K. pneumoniae (49.5%) and S. aureus (41.8%). The high incidence of Ps. aeruginosa was consistent with the incidence on burn wound cultures and biopsies. It was evident that, in this period, a close correspondence between wounds and I.V. catheter flora existed, at a period when sepsis was predominantly due to Klebsiella. The Pseudomonas incidence was most plausibly a reflection of burn wound colonization flora.

Table 16. Bacterial Flora of I.V. Catheter Tips
from 170 Burn Patients, 1975

Species	No. Patients Positive	% of All Patients Positive	% of Patients With Positive Cultures
<i>S. aureus</i>	38	22.3	41.8
<i>S. epidermidis</i>	12	7.0	13.1
Alpha.hemol.-strep	1	0.6	1.1
Beta hemol.-strep	2	1.2	2.2
Non-hemol.-strep	14	8.2	15.4
<i>Bacillus</i> sp.	7	4.1	7.7
<i>Ps. aeruginosa</i>	50	29.4	55.0
<i>Mima-Herellea</i> gp.	0	0	0
<i>K. pneumoniae</i>	45	26.5	49.5
<i>Entero. cloacae</i>	17	10.0	18.7
<i>S. marcescens</i>	7	4.1	7.7
<i>E. coli</i>	6	3.5	6.6
<i>Prot. mirabilis</i>	5	2.9	5.5
<i>Prov. stuartii</i>	5	2.9	5.5
<i>Candida</i> sp.	10	5.9	11.0
<i>Citrobacter</i> sp	1	0.6	1.1

No. catheter tips cultured: 471
Average catheter tips per patient: 2.7
No. of Patients with Positive Cultures: 91

DISCUSSION

The period reviewed in this summary coincided with a major epidemiologic change in the nature of burn wound infection in the ISR. S. aureus remained, as it had in recent years, an important part of the infection flora in burns in this Institute. But the most lethal organism, and the cause of a major part of sepsis in the severely burned, was K. pneumoniae. This opportunistic enteric species succeeded Enterocloacae as the predominant opportunist in setting up infection which could lead to sepsis. No other gram-negative species incited an epidemic of sepsis in 1975. Ps. aeruginosa, however, became more prominent than it had in the recent past, especially in burn wound tissue. The lessened anti-pseudomonal activity observed in wounds topically treated with silver sulfadiazine compared with burns topically treated with Sulfamylon would appear to be an explanation for this rise in incidence of this ubiquitous burn wound colonizer. The virtual disappearance of Prov. stuartii and Enterocloacae as wound invading forms were the most striking epidemiologic events in 1975. The possibility that other enteric species may emerge as opportunistic pathogens in the future is real. Antibiotic susceptibility of most of these forms makes them potentially susceptible to chemotherapy, but experience with sepsis due to Enterobacteriaceae does not offer an encouraging prospect for future control of infection in burns.

PUBLICATIONS

Lindberg RB, Pruitt BA, Jr, Mason AD, Jr: Topical Chemotherapy and Prophylaxis in Thermal Injury. Chemotherapy vol. 3, 1976, pp. 351-359.

PRESENTATIONS

Lindberg RB. Topical chemotherapy and the treatment of burns. IX International Congress of Chemotherapy, London, England. July 26, 1975.

Lindberg RB: Summarizing comments and session on Topical Chemotherapy of Wounds. IX. International Congress of Chemotherapy, London, England. July 27, 1975.

Lindberg RB: The microbiology of severe burns. Birmingham Accident Hospital, Birmingham, England. Surgical and Medical Staff Seminar. July 30, 1975.

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<p>23. (U) <u>Pseudomonas aeruginosa</u> has shown increasing potential for producing nosocomial infection and despite intermittent effective control, remains ubiquitous in burn patients. Control of such infections is a primary objective of combat medicine and only with a precise knowledge of strain identity can it be achieved or documented.</p> <p>24. (U) Phage typing procedures, plus modifications to establish an identification system in the non-typable strains were applied to all Pseudomonas isolates.</p> <p>25. (U) 75 07 - 76 09 Strains typed with RTD made up 26% of isolates; 74% could be differentiated by lysis with undiluted phage. As in 1974, four epidemic types predominated at different periods in 1975, but each one was a new type--not present in 1974. The typing system delineated epidemic patterns and permitted precise recognition of virulent strains which were sought for further evaluation as to the relative role of virulence in human pathogenesis.</p>							

^a Available to contractors upon originator's approval

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TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE - SURGERY

**REPORT TITLE: BACTERIOPHAGE TYPES OF PSEUDOMONAS AERUGINOSA FOUND
IN BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROCKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 June 1976

Investigators:

**Ruth L. Latta, BS
Robert B. Lindberg, PhD
Arthur D. Mason, Jr, MD
Basil A. Pruitt, Jr, MD, Colonel, MC**

Reports Control Symbol MEDDH-288 (R1)

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ABSTRACT

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Continued application of dilute and concentrated bacteriophages of the ISR Pseudomonas aeruginosa phage typing system has made possible the characterization of 95% of isolates from burn patients in 1975. Ps. aeruginosa infection involved increasing numbers of patients annually for the past 3 years. In 1975, 1561 strains were collected from 161 patients. Type NT-16, (21, 68, F7) was the most prominent epidemic type, continuing from 1974, when it appeared abruptly in July. The second most important type, E-2, (24) had previously been seen only as a rare type. Types predominant in septicemia and in lung tissue at autopsy included strains typical of the overall type distribution and also strains which were relatively more common in blood and in lung tissue than in the Pseudomonas population as a whole. Epidemic episodes extended over periods from 8 weeks to 2 years, depending on the type causing infection.

Pseudomonas
Phage typing
Burn wound
Topical chemotherapy
Humans

BACTERIOPHAGE TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

Pseudomonas aeruginosa has shown a progressive capacity for extension as a nosocomial pathogen in a variety of disease states, and on a world-wide basis. This extending involvement is particularly disturbing in view of the concomittant development of antimicrobial agent resistance of Ps aeruginosa; it has become increasingly apparent that individual successes have not altered the potential gravity of Pseudomonas infections in hospitals.

Burn wound infections are one of the most serious problems caused by Ps aeruginosa, and their role in pathogenesis of infection during burn treatment has remained prominent despite development of effective topical therapeutic and systemic agents (1,2). A burn ward such as that of the US Army Institute of Surgical Research offers significant advantage in studying the Pseudomonas problem: The proportion of patients with severe burns, at high risk of developing invasive sepsis, is large, and proximity presents a dismayingly optimal circumstance for transmission, so that strains capable of setting up an epidemic situation are offered the opportunity to do so. Precise recognition of bacterial strains of an infecting species is essential if the epidemiology of an ongoing infection problem is to be assessed. This differentiation not only permits precise characterization of infections that occur, but makes possible the selection of strains which vary in virulence, invasiveness, and response to treatment. Phage typing was selected as the method of choice for this differentiation (3,4), and a definitive set of phage for this procedure was developed in this laboratory and has been in use for 15 years. The phages have been furnished to other workers throughout the world, in England, Denmark, Japan, Germany, and Australia, and the value of the system has now prompted the Center for Disease Control (Atlanta) to request a set of phages to be used as an international definitive typing set.

-
1. Lindberg RB, Moncrief JA, Switzer WE, Order SE, Mills W, Jr: The successful control of burn wound sepsis. J Trauma 5:601-616, 1965.
 2. Curreri PW, Lindberg RB, DiVincenti FC, Pruitt BA, Jr: Intravenous administration of carbenicillin for septicemia due to Pseudomonas aeruginosa following thermal injury. J Inf Dis (Suppl) 122:S40-S43, 1970.
 3. Lindberg RB, Latta RL, Brame RE, Moncrief JA: Definitive bacteriophage typing system for Pseudomonas aeruginosa. Bact Proc 1964, p.81.
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METHOD

Previous reports have presented the recognition of the ongoing problem of the appearance of strains of Ps aeruginosa which did not react with the Routine Test Dilution of some bacteriophages. These non-typable (NT) strains react with undiluted typing fluids, and to further the objective of reproducibility and continuity of results, undiluted typing was performed on strains NT at RTD. The prefix "NT" has continued to be used as the designation for the type of such strains, with no evaluation made for individual strains as to whether or not such lysis constitutes a pyocin reaction as opposed to a phage lysis.

Phage typing of Pseudomonas aeruginosa from Burn Ward Patients, 1975

The number of patients from whom Ps aeruginosa was recovered has increased in recent years as shown in Table 1.

Table 1. Pseudomonas aeruginosa recoveries
in ISR, 1973 - 1975

Year	No Patients admitted	No Patients from whom <u>Ps aeruginosa</u> recovered	No. Strains <u>Ps aeruginosa</u> Collected for Phage Typing
1973	256	97	788
1974	244	116	1085
1975	245	161	1561

During 1975, 1561 strains of Ps aeruginosa from 161 patients on the burn wards were collected for phage type identification. Sources included blood, wound surface, biopsy, catheter, sputum, urine and other sources.

Predominant phage types of strains typable either at RTD or with undiluted phage, are shown in Table 2. The Phage Type Code (left column) shows the assigned letter-number designation which has been developed to facilitate characterizing any given phage sensitivity pattern. Recall that "NT" designates strains not typable with dilute, but typable with concentrated phage. The phage type code lists the phages reacting; the NT designation shows that the reaction occurs only with undiluted phage. These patterns are marked with an asterisk (*). The proportions of patients harboring one of the principal types is greater than 100%, because of multiple types being found on one patient. In terms of proportion of strains belonging to a given type, the 9 patterns which were distinguished

included 84.7% of all strains tested. The remaining 15.3% of strains were distributed over a large number of minor types. Any types found in fewer than 8 persons were not included; this practice prevents the inclusion of an unwieldy number of strains of minor numerical importance.

Table 2. Predominant Phage Types of 1,561 *Pseudomonas* Strains from 161 ISR Burn Ward Patients, 1975

Phage Type Code	Phage Type	Patients	Strains
		Per Cent	
NT-16	Non-typeable * (21), 68, (F7)	36.6	20.0
E 2	24	33.5	17.0
NT-19	Non-typeable * (21), 24, 44, 1214, 68	30.4	11.8
NT-22	Non-typeable * 44, 1214, 68, (109), 352, F8	29.2	18.0
H 1	1214	14.9	7.4
NT-24	Non-typeable * (31), 68	11.2	5.3
E 2 ^v	24 or Non-typeable * (24)	10.5	2.4
NT-3	Non-typeable * (21), (24), (44), 1214, (68), 109, 352, (F7), F8	4.9	2.0
NT-23	Non-typeable * (21), 68	4.9	0.9

* Phage Type using undiluted phage
() Variable reaction

Nine separate types of *Ps aeruginosa* played major roles in burn wound infection during 1975. Three types, representing 58.9 of the strains listed, reacted at the RTD; the remaining 6 types were recognized only in undiluted (NT) reaction. In the case of E-2 and E-2^v, two distinct levels of reactivity were observed with these two strains. They are entered as two distinctive types in view of morphologic differences in reaction. E-2^v strains fluctuated in their reaction with phage type 24, while E-2 strains were consistent in level of sensitivity and in exhibiting a characteristic plaque reaction in RTD typing.

The most prevalent strain of *Ps aeruginosa* 1975 was NT-16, reacting with 21, 68, F7 phages. This was a relatively new type, first observed

in July 1974. It rapidly occupied a predominant position on burn patients and in 1975, was the most pervasive strain, on 36.6% of the patients positive for *Pseudomonas*, and with one-fifth of all isolates typed.

Phage type E-2, reacting with phage 24, was the second most common type in 1975. This type had been observed as a rare type in earlier years, although at least since 1970 it had never been common enough to merit inclusion in types recovered. None had been observed during 1974.

Type NT-19, the third most frequent type, involved a high proportion of patients (30.4% of all those with *Pseudomonas* present), but presented fewer strains than did the next most common type. This too was a strain which increased strikingly from almost zero incidence; only two strains were collected in late 1974.

Type NT-22 was not seen at all in 1974. It appeared abruptly in July 1975, spread very rapidly, and became fourth in incidence for the year.

The first four categories included 66.8% of all strains collected, and thus embodied the major part of the epidemic spectrum of *Ps aeruginosa* for 1975.

Type 12-14 or H-1, which had been observed in earlier years but not in 1974, first appeared in May 1975. 14.9% of patients harbored it, and 7.4% of all strains collected were of this type.

Type NT-24 was a new type, never previously observed. It appeared in September 1975, and thus spread very rapidly to colonize the ward as the sixth most common strain for the year. The pattern is related to 31 and to 68, but these reactions were never previously linked.

Phage type E-2^V was described above. It reached 10.5% of patients, and was a new previously unrecorded type.

The last strains recorded were NT-3 and NT-23, each was found in 4.9% of the patients. NT-3 was more common among total strains but still made up only 2% of isolates typed. In the previous year, 1974, NT-3 was found in 32.75% of patients, but declined rapidly in incidence in 1975. NT-23, (21) 68, was another new type, never previously observed.

Thus, there were 9 types predominant in 1974, of which 7 had not been seen or were of negligible incidence in 1974. This was an unusually high rate of incursion of new types into the ISR burn ward, but showed how rapidly new strains can pervade a burn population such as the one presented here. 84.8% of all strains fell in one of the 9 types listed, and 66.8% of all strains tested in 1975 were NT in contrast to 75% in 1974. In that year, the predominant population included 12 types; the pattern in 1975 was consistent with more permeation by individual epidemic

strains. The validity of the typing system for characterizing *Pseudomonas* strains was re-affirmed, and the undiluted typing addition appeared to be valid.

Phage Types of *Pseudomonas* Recovered in Blood of ISR Burn Patients, 1975

Aside from general seeding of most burn patients, the role of *Pseudomonas* in sepsis is of great importance, since such septicemia displays a very high mortality rate, despite use of antibiotics which may be effective in vitro. Forty-five burn patients with positive blood cultures contributed 91 strains for typing. Phage types observed are listed in Table 3. Note that the most common phage types, among the total of strains collected, are represented among the five most common blood stream types. In terms of totals of patients from whom these types were recovered, NT-16 was more common in the total than in blood stream isolates, by 36.6% to 15.5%. Similarly, NT-19 was recovered from 30.4% of all patients, but only from 4.4% of blood culture positives. There were no strains which were more common in blood than in the population at large. The pattern of sepsis, however, in relation to chronologic sequence, is better shown by detailed presentation, as shown in Table 4. It was obvious that the successive episodes of septicemia were not scattered; instead, the various types were clumped within given time periods, reflecting the epidemic nature of the episode the patients are represented by arbitrary accession numbers. Those patients who had a given type recovered in successive months are marked with an asterisk. The principal 5 phage type codes are listed, plus a column for those types occurring only in one or two patients.

Table 3.
Phage Types of *Pseudomonas* Strains from Blood Stream
of 45 ISR Burn Ward Patients, 1975

Phage Type Code	No. of Patients	No. of Strains
NT-22	16	25
E 2	15	31
NT-16	7	13
E 2'	4	4
H 1	3	6
B22 NT-17 NT-19	2	3
NT-3 NT-23 NT-	1	1

Table 4. Phage Types of 91 Pseudomonas Strains from Blood Stream of ISR Burn Ward Patients, 1975

Month	Patient No.	No. of Strains	Phage Type Code					
			E 2	E 2 ^v	H 1	NT-16	NT-22	Other
			No. of Strains					
Jan	223 '74	1						NT3-1
	240 '74	3				3		
	242 '74	1				1		
	3	2						NT17-2
	4	1						NT17-1
Feb	6	1				1		
	23	1						NT19-1
	27	3	2			1		
	28	1						NT -1
Mar	33	2	2					
	38	3	3					
	41	1						B22-1
	48	1	1					
Apr	46	3	1			2		
	53	2	2					
	56	1	1					
	57	2	2					
	62	5	4			1		
	63	4	4					
	68	1	1					
	69	3	3					
	79	4	2	1		1		
	80	1	1					
May	*79	3				3		
	*80	1	1					
	89	1	1					
	98	1		1				
Jun	59	1		1				
Jul	122	1		1				
	137	1					1	
Aug	144	3			3			
	151	1			1			
	156	2			2			
	163	2						NT19-2
	168	1					1	
Sep	*163	4					1	B22-2
	176	2					2	NT23-1
	179	1					1	
	186	1					1	
Oct	*151	2					2	
	189	3					3	
	193	1					1	
	196	1					1	
Nov	*196	2					2	
	204	1					1	
	207	1					1	
	208	4					4	
Dec	218	1					1	
	221	1					1	
	227	1					1	

Type NT-22 was the most frequent septicemic strain occurring from July through December. The strain was fourth in frequency from all sources. Type E-2 (phage 24) was the second most frequently observed, and involved 15 septicemic patients from February through May. Third in incidence was type NT-16, which was the most common strain recovered from all sources, its incidence, from January through May, paralleled that of type E-2. From April through July, the related type E-2^V was recovered in 4 patients. The implication that E-2^V was a loss variant of E-2 is strongly suggested by this sequence. Type H-1, found in 3 patients, appeared only in August, in blood cultures. The six least common types, B-22, NT-17, NT-19, NT-3, NT-23 and a single non-reactive strain, were found from January to March, then in August and September. Only one, NT-17, appeared in two cases in close succession.

There were, thus, 91 strains of 11 phage types recovered from blood. No apparent correlation of phage type (or strain) with septicemia was apparent. Instead, the invasive behavior was roughly a reflection of the overall incidence of Ps aeruginosa in the population.

Pseudomonas Phage Types Recovered From Lung Tissue of ISR Burn Patients at Post-Mortem, 1975

Pseudomonas strains from lung tissue at autopsy furnish one of the principal sources of definitive information about the pneumonia which so frequently occurs as part of the pathologic problem in the burn patient. Phage types of these strains, in comparison with septicemic strains, present an insight into the source of systemic invasion in these patients. Phage types of Pseudomonas from lung tissue are summarized in Table 5. The frequency of occurrence of types was not entirely that seen in the overall population nor was it identical with that of blood stream isolates. Type E-2 was far less common than it was in blood or wound type NT-22 was most common in both blood and lung, but was less common on wounds and other sites.

Comparative frequency of occurrence of principal phage types in blood, and lung and in the entire collection from all sources is summarized in Table 6.

As with septicemias, a more adequate picture of epidemiology is seen when the chronologic sequence of type incidence is shown. This pattern is presented in Table 7. The most common phage types are shown individually; additional infrequent types are shown in the month of their occurrence.

The most common epidemic type, NT-22, was found in lungs of 16 patients between August and December. Type NT-16, found in 13 patients, was recovered in 13 patients from January through July. These two strains were in succession predominant for the entire year. Type NT-19, the third most frequently seen, was found in 9 patients from January through August, and then once more in December. NT-24 was recovered from lungs of 5 patients from August through November. The less frequent types included E-2,

Table 5.
Phage Types of Pseudomonas Strains from Lung Tissues
of ISR Burn Ward Patients, 1975

Phage Type Code	No. of Patients	No. of Strains
NT-22	16	30
NT-16	13	25
NT-19	9	19
NT-24	5	9
E 2	3	7
H 1	2	7
NT-3		2
NT-17		5
Other	10	14

no. of patients: 41

no. of specimens: 102

no. of strains isolated: 118

Table 6.
Relative frequency of Occurrence of
Ps aeruginosa Phage Types, ISR 1975

Type	Frequency & Source		
	All Sources	Blood	Lung
NT-16	1	3	2
E-2	2	2	5
NT-19	3	6	3
NT-22	4	1	1
H-1	5	5	6
NT-24	6	0	4
E-2 ^v	7	4	0
NT-3	8	7	6
NT-23	9	7	0

Table 7. Phage Types of 118 Pseudomonas Strains from Lung Tissues of ISR Burn Ward Patients, 1975

Month	Patient No.	No. of Specimens	Phage Type Code						
			E 2	H 1	NT-16	KT-19	NT-22	NT-24	Other
			No. of Strains						
Jan	223 '74	3			1	2			D41-1 NT3-1
	240 '74	3			3				
	3	4			3	3			NT17-1 NT17-4 F 5-1
	4	4							
Feb	15	1							
	6	1			1				
	23	2				2			
	25	4	1		1	2			NT -2 NT -2
Mar	27	3				2			
	28	2							
Apr	33	4			4				
Apr	46	3			2				NT3-1
	53	1			1				
	57	2			2				
May	79	3			3				
	80	4	4						
	83	1			1	1			
	85	1			1				
Jun	109	2	2						NT -1
Jul	137	4		4	2				
Aug	144	4		3		4			NT23-3
	156	4				2	2		G28-1
	166	3							
Sep	163	4					4		
	168	3					3		
	176	3					3		
	183	1					1		
	186	2					2		
Oct	151	2						2	
	189	3					1	2	
	190	1					1		
	197	4					3	1	NT -1
	199	3					2		C23-1 NT -1
	202	1					1		
Nov	205	1					1		
	201	1					1		
	207	1							
Dec	214	3						3	
	221	2					1		
	227	3					3		
Dec	240	1				1			

found in lungs of 3 patients in February, May, and June. Two patients each exhibited H-1, NT-3 and NT-17, and 10 additional types were each found in only one patient. Obviously, there was no one type causing pneumonia, but there were significant differences in septicemia and pulmonary phage types. The NT-16 epidemic period for both sources was the same, from January to May or July. Type NT-22 also appeared at the same time interval for both sources. But the E-2 type found extensively in blood cultures from February through May was seen in lungs of only 3 patients during this period. Type NT-19, prominent from January to August, was found in the blood of only two patients. Thus a major episode of bacteremia was caused by a type rare in the lung, while a pulmonary strain of extensive occurrence was rare in the blood.

An attempt to assess differences in incidence of phage types by location was made in view of the differences in incidence thus far noted. Figure 1 compares the incidence of strains from blood, lung tissue, and all other sources. The proportionate incidence of the major types is represented for each source by the distinctive bar form. Type NT-16 was most common in lung and all other sources, but far less often recovered from blood. Type E-2 was found equally often in blood and all other sources, but was relatively uncommon in the lung. NT-19 was relatively rare in blood, but almost as frequent in lung as in other sources. Type NT-22 was most common in lung, least frequent in sources other than blood. The remaining principal types, H-1, NT-24, E-2^V, NT-3 and NT-23, were all less common than the first 4. H-1 was most common in other sources, least common in blood. Type NT-24, surprisingly, was never found in blood culture. Conversely, E-2^V was not found in lung. NT-3 was least often found in blood, but both NT-3 and NT-23 were the least common of the major types recognized.

Monthly Distribution of Prevalent Phage Types, ISR, 1975

The occurrence of small monotype epidemics of Ps aeruginosa has been documented in previous years, but the continued change in types, and the appearance of previously unrecorded types makes this observation of succession of types a continuing important assessment. Figure 2 summarizes the monthly incidence of 11 major phage types of Ps aeruginosa. All but two strains involved at least 8 patients. Two strains involved only 3 patients each. To render the distribution more legible, the most prevalent type for each month is outlined in solid black, the next most prevalent by a double line, and third most common by a single line.

The first 5 types listed, NT-17, NT-18, NT-3, NT-19 and NT-16 were types that had been seen in 1974. In that year, NT-18 was 10th in frequency, while NT-3 was the most common type found in 1974. Conversely, only two strains of NT-19 were found in 1974. NT-16, which like NT-19 has been a major type in 1975, was also the second most common type found in 1974. NT-17 disappeared after its appearance in January. NT-18

Figure 1. Incidence of Predominant *Pseudomonas* Phage Types from Blood, Lung Tissues, and Other Sources

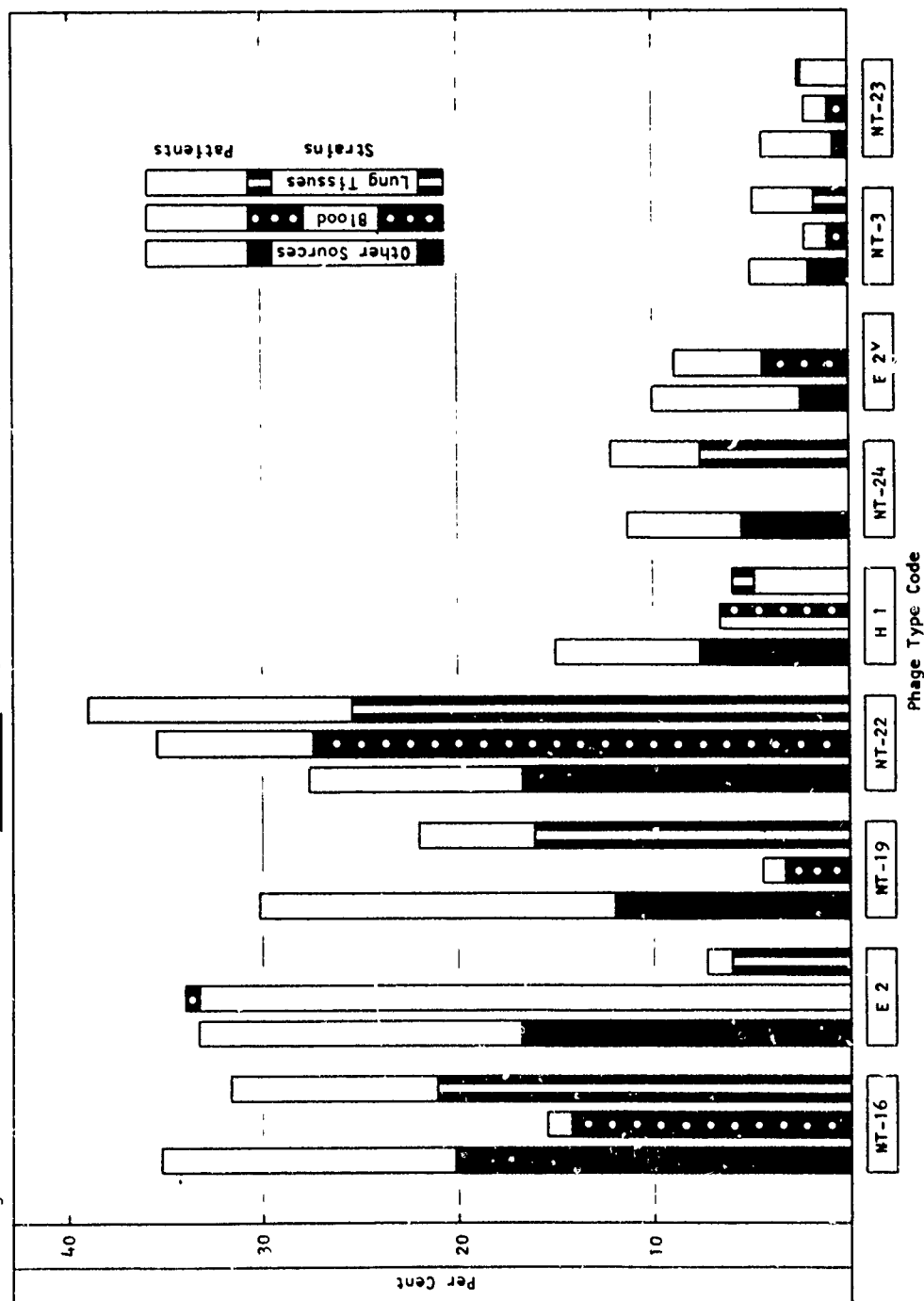


Figure 2. Monthly Distribution of Predominant Pseudomonas Phage Types in ISR Burn Ward Patients, 1975

Phage Type Code	Month												Total Patients-Strains Each Type
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
NT-17	3-12												3-12
NT-18	2-10	3-7		1-1									3-18
NT-3	4-24	4-5		1-2									8-31
NT-19	4-25	7-53	6-20	9-16	4-16	2-2	2-2	10-32	3-4	4-5	2-5	3-4	49-184
NT-16	10-46	9-50	8-30	18-79	9-62	5-7	6-19	5-10	2-3	2-3	1-3		59-312
E 2		5-21	15-63	23-114	11-43	9-20	2-4						54-265
E 2v				6-9	4-6	5-10	2-4	1-6	1-2				17-37
H 1					1-11	1-2	5-13	14-58	5-11	4-20	1-1		24-116
NT-22							4-16	5-7	11-64	14-79	16-69	10-46	47-281
NT-23								4-8	2-3	2-2	1-1		8-14
NT-24									1-1	9-32	9-37	4-13	18-83
Total Patients-Strains Each Month	18-167	17-168	18-136	30-246	18-139	16-50	17-65	23-137	18-98	18-154	22-126	17-75	

similarly was seen only briefly in early 1975; NT-3 disappeared after April 1975.

NT-19 was a prominent feature of the burn flora in 1975; it was recovered every month, although it was never a major type. Type NT-16 was recovered in 11 of 12 months in 1975, and was predominant during 3 months. Its frequency was diminishing during the last quarter of 1975, and it can be projected that it may disappear.

Type E-2 presented an acute 4 months outbreak in March through June, when it was the dominant type. The closely related E-2' was recovered over a 6 month period, but was only once the second most common type.

Type H-1 displayed a capability for rapid dominance of the patient, flora especially in August when it was the predominant type. It persisted over a 7 month period, then disappeared abruptly, as it had entered the burn ward population. NT-22 strains appeared abruptly in July, increased in incidence from 4 to 5 patients, then assumed the predominant status on the burn ward through December. Type NT-23 occurred during a 4 month period starting in August but was never prominent. The last type NT-24, appeared in September, then for the rest of the year was the second most common type. Numerically it would be regarded as an epidemic strain; only the preponderance of NT-22 overshadowed its frequency.

The pattern of major and lesser epidemics of Ps aeruginosa appearing for larger or shorter periods was evident in this period of observation, as it had been in previous years. The second most common epidemic type, NT-19, had appeared in only 2 isolates in 1974; it was conspicuous but never predominant throughout 1975. Type NT-16 had been the second most common type in 1974. Type E-2 typifies the previously unknown type that appeared with explosive suddenness. There is still no explanation for the disparate behavior among strains of Ps aeruginosa in a burn ward.

PUBLICATIONS

None

PRESENTATIONS

Lindberg RB: "Epidemiology of Pseudomonas aeruginosa strains in nosocomial infections." Presented at Am Pub Health Assoc. Symposium, New Orleans, 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3 DATE PREV SUMMARY 75 07 01	4 KIND OF SUMMARY H. TERM	5 SUMMARY SCTY ^a U	6 WORK SECURITY ^a U	7 REGRADING ^a NA	8A DISB'N INSTR ^a NL	8B SPECIFIC DATA - CONTRACTOR ACCESS <input type="checkbox"/> YES <input type="checkbox"/> NO	9 LEVEL OF SUM A. WORK UNIT
10 NO./CODES ^a		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY		61102A	3A161102B71R	01		243	
b. CONTRIBUTING							
c. CONTRIBUTING							
11 TITLE (Precede with Security Classification Code) ^a (U) Bacteriophage Types of Serratia Marcessens From Burn Wounds of Military Personnel (44)							
12 SCIENTIFIC AND TECHNOLOGICAL AREA ^a 003500 Clinical Medicine							
13 START DATE 67 07		14. ESTIMATED COMPLETION DATE 76 09		15. FUNDING AGENCY DA		16 PERFORMANCE METHOD ^a C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING 76		.6	
b. NUMBER ^a				FISCAL 77		.3	
c. TYPE.				CURRENT		9	
d. AMOUNT:							
e. KIND OF AWARD.				f. CUM. AMT.			
19 RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Virginia E. English, MA			
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Burns; (U) Serratia; (U) Bacteriophage; (U) Humans							
23 TECHNICAL OBJECTIVE, 24 APPROACH, 25 PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
23. (U) A potential epidemic pathogen of significance, <u>Serratia marcessens</u> , has been observed in periodic outbreaks in burn patients. Transmission appears to be relatively rapid. Since such opportunists can present serious hazards to severely burned soldiers, a means of strain differentiation was sought by development of a phage typing system, as a direct major tool in control of such opportunistic pathogens.							
24. (U) Phages, recovered from sewage effluent, were propagated to derive high titer distinctive reactions capable of differentiating strains of <u>Serratia marcessens</u> .							
25. (U) 75 07 - 76 09 Although the previously reported phage typing set was capable of differentiating <u>Serratia</u> epidemic strains successfully, instability of a major component phage in the system necessitated further phage isolations and differentiation by reaction patterns. A more definitive typing system evaluated in terms of the previous experience, has resulted, and this improved system is undergoing intensive evaluation to confirm its differentiating capability.							

^aAvailable to contractors upon originator's approval

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 66 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

**REPORT TITLE: BACTERIOPHAGE TYPES OF SERRATIA MARCESCENS FROM
BURN WOUNDS OF MILITARY PERSONNEL**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Robert B. Lindberg, PhD
Virginia C. English, MA
Basil A. Pruitt, Jr, MD, Colonel, MC
Arthur D. Mason, Jr, MD**

Report Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: BACTERIOPHAGE TYPES OF SERRATIA MARCESCENS FROM
BURN WOUNDS OF MILITARY PERSONNEL

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Robert B. Lindberg, PhD
Virginia C. English, MA
Basil A. Pruitt, Jr, MD, Colonel, MC
Arthur D. Mason, Jr, MD

Report Control Symbol MEDD-H-288 (R1)

The phage typing set for Serratia marcescens has been re-assessed to avoid problems associated with phage viability and the specificity of typing patterns. New phages have been collected to remedy these deficiencies, and a comprehensive typing will be applied to strains of S. marcescens collected for this assessment.

The importance of a technic for differentiating strains of S. marcescens is apparent in view of the capacity of this enteric species to cause severe nosocomial infections, including pneumonia, infections in surgical wounds and in thermally injured patients. To achieve precise strain recognition, a phage typing system was developed in the ISR laboratory.

The typing set of 7 phages was applied to differentiating Serratia isolates from ISR wards and from other hospitals. The system was effective, and phage types were distinguishable. The technical approach was valid. However, the phages selected included some which were exceptionally labile, and which could fail to survive storage at 4°C, the usual methods of holding bacteriophages. Further, the phage type pattern achieved with this typing set, although usable, revealed a coverage of wild strains that was less definitive in making type distinctions than had been desired.

In view of these factors, re-study and acquisition of additional phages has been carried out during this interval. New phages have been recovered, propagated and compared for identity with the existing phage collection. An improved typing set is in process of being selected and standardized. It will be assessed on S. marcescens collections now held for this purpose.

Burns Serratia Bacteriophage Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY					1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL
					DA OE 6382	76 10 01	DD-DR&E(AR)636
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DES'N INSTR ^a	9. SPECIFIC DATA- CONTRACTOR ACCESS	10. LEVEL OF SUM A. WORK UNIT
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NC / CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61102A		3A161102B71R		01	
b. CONTRIBUTING						317	
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Five Per Cent Aqueous Sulfamylon Soaks Used in Topical Treatment of Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 10		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
Not Applicable				PRECEDING			
a. DATES/EFFECTIVE:				76			
EXPIRATION:				.4			
b. NUMBER ^a				77			
c. TYPE:				.1			
d. KIND OF AWARD.				FISCAL YEAR			
e. CUM. AMT.				CURRENT			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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ADDRESS ^a Fort Sam Houston, Texas 78234				Burn Study Branch			
				Fort Sam Houston, Texas 78234			
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FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Daryl R. Erickson, MD			
				NAME: Barry A. Levine, MAJ, MC			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Burn; (U) Eschar separation; (U) 5% Sulfamylon acetate solution; (U) Humans							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) The objective of this study is to demonstrate the efficacy of 5% Sulfamylon acetate solution as an effective topical antibacterial agent when used either as continuous soaks or wet to dry dressings during the latter stages of eschar separation and when used as continuous soaks covering mesh grafted areas in soldiers with burn injury.							
24. (U) Five percent Sulfamylon acetate is used as a debriding agent by applying gauze sponges soaked in a solution to the burn wound and wrapping the area. The gauze sponges are applied saturated with the solution and they are allowed to dry and removed every six to eight hours. The five percent Sulfamylon acetate solution is also used to keep dressings covering mesh graft wet until there is adherence of the mesh graft. This markedly decreases the bacterial flora.							
25. (U) 75 07 - 76 09 Five percent Sulfamylon acetate solution was used in 128 patients. In 119 cases it was used with wet to dry applications of coarse mesh gauze to facilitate debridement. In 106 cases it was used in conjunction with mesh grafting. There were two cases of severe respiratory insufficiency among the 128 patients treated giving a complication incidence of 1.6%.							

^a Available to contractors upon originator's approval

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1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A, 1 NOV 65
AND 1498 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

**REPORT TITLE: FIVE PER CENT AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL
TREATMENT OF BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Hugh D. Peterson, DDS, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: FIVE PER CENT AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 Jul 5 1975 - 30 September 1976

Investigators: Hugh D. Peterson, DDS, MD, Col, MC
Basil A. Pruitt, Jr., MD, Col, MC

Reports Control Symbol MEDDH-288(R1)

Five per cent Sulfamylon acetate solution was used in 128 patients as the wetting agent in the wet to dry coarse mesh gauze debridement technique and for continuously wet dressings used to cover excised and grafted areas in the burned soldier. Fifty-two point two per cent of all admissions had the agent used at least for one of the above mentioned techniques. Most of these patients (84 per cent) had the five per cent Sulfamylon acetate solution used for both treatment modalities. Toxic reactions manifested by pulmonary and/or cerebral signs were clinically diagnosed as being related to the use of 5% Sulfamylon acetate solution in two cases. Five per cent aqueous Sulfamylon soaks offer excellent control of the bacterial population of either separating eschar or healthy granulation tissue.

Burn
Eschar separation
5% Sulfamylon acetate solution
Humans.

FIVE PER CENT AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

Five per cent Sulfamylon acetate solution was used in 128 patients or 52.2 per cent of all admissions. It was used on 119 patients as the fluid agent in wet-to-dry coarse mesh gauze dressings used for wound debridement. It was used as an antibacterial wetting agent for continuous wet dressings covering meshed (graft) or excised areas in 106 patients. Most of these patients (84 per cent) had the five per cent Sulfamylon acetate solution used for both treatment modalities.

Toxic reactions manifested by pulmonary and/or cerebral signs were clinically diagnosed as being related to the use of five per cent Sulfamylon acetate solution in two cases. The pulmonary problems continued to be principally hyperventilation with associated mental confusion in some patients, resolving with removal of the topical soaks.

Since five per cent Sulfamylon solution is generally used 2 - 3 weeks post burn except in the case of excisions, it appears that when not combined with Sulfamylon cream the patients tolerate it extremely well.

Five per cent aqueous Sulfamylon soaks offer excellent control of the bacterial population of either separating eschar or healthy granulation tissue, with minimal toxicity.

PRESENTATIONS AND/OR PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OB 6982	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DES'N INST'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61102A	3A161102B71R		01	223		
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Development of Prophylactic Topical Therapy For Use on Burn wounds of Military Patients: Search For Improved Formulations (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
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b. NUMBER: ^a				76		.6	
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e. KIND OF AWARD:		f. CUM. AMT.		CURRENT		.2	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a US Army Institute of Surgical Research				NAME: ^a US Army Institute of Surgical Research			
ADDRESS: ^a Fort Sam Houston, Texas 78234				ADDRESS: ^a Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish NAME if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: ^a Robert B. Lindberg, PhD			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2018			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
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				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Burns; (U) Sulfamylon; (U) Semi-synthetic penicillins; (U) Pseudomonas; (U) Rats							
23. TECHNICAL OBJECTIVE ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number Precede last of each with Security Classification Code)							
23. (U) Assessment of virulence of new epidemic types of <u>Pseudomonas aeruginosa</u> has been called for by appearance in the burn ward of previously unknown strains varying in virulence and in response to chemotherapy. This assessment and evaluation of new antimicrobial agents, an essential aspect in preparing for the management of thermal injury in troops in combat.							
24. (U) Surveillance of infecting types to include virulence assessment of each epidemic form and experimental therapy on challenged animals.							
25. (U) 75 07 - 76 09 Epidemic strains varying in virulence from 15% to 90% in seeded burned rats were uncovered. Type A-71, a recurrent epidemic type, was virulent for 9.9% to 100% of animals. In its complete form, it was treatment refractory in animals. Pirbenicillin was tested for control of burn sepsis. Results indicated partial effectiveness.							

^a Available to contractors upon originator's approval

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: DEVELOPMENT OF PROPHYLACTIC TOPICAL THERAPY FOR
USE ON BURN WOUNDS OF MILITARY PATIENTS: THE
SEARCH FOR NEW FORMULATIONS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

Robert B. Lindberg, PhD
Virginia C. English, MA
Ruth L. Latta, BS
George T. Daye, MA
Basil A. Pruitt, Jr, MD, Colonel, MC

Report Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: DEVELOPMENT OF PROPHYLACTIC TOPICAL THERAPY FOR
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The ultimate criterion for improved chemotherapy of cutaneous burns is based on reliable animal models. The burned rat seeded with Pseudomonas aeruginosa offers the most plausible simulation of human burn infection, but detailed study of a series of wound and blood stream isolates from Pseudomonas sepsis revealed a spectrum of inherent variation in virulence from zero to 100%. Strain variation occurs with many types, but a major epidemic phage type was recovered over an 8 year period with no change in virulence. In contrast, successive isolates during prolonged clinical sepsis in a major burn revealed inherent, stable variations of a major order in virulence and response to therapy of specific types. Surface tension alteration was shown to lack significance in affecting virulence or invasiveness. A trial of a new semi-synthetic penicillin, pirbenicillin, showed it to be ineffective in control of experimental Pseudomonas burn wound sepsis.

Burns
Pseudomonas
Sulfamylon
Semi-synthetic penicillins
Rats

DEVELOPMENT OF PROPHYLACTIC TOPICAL THERAPY
FOR USE ON BURN WOUNDS OF MILITARY PATIENTS
THE SEARCH FOR NEW FORMULATIONS

The assessment of virulence in Pseudomonas aeruginosa has been based on the ability of strains of this organism to set up invasive burn wound sepsis in the white rat when a burn is seeded with a young culture. The disease which results is strikingly similar to burn wound sepsis in the human, and medication designed to control this syndrome in animals has been highly effective on human subjects. Apart from the practical aspect of an infection model in deriving new modes of therapy, the seeded burned rat model offers an important epidemiologic tool for determining the extent of virulence in Ps. aeruginosa, the relationship of virulence to spread and persistence of a given strain in a patient population, the detection of virulence-related attributes in pathogenic strains, and other basic criteria of importance in understanding wound infection. Since the occurrence of epidemic waves of Ps. aeruginosa infections due to specific phage types has been well documented, there is ample reason to assess strains which cause invasive sepsis. These will vary, not only as to identity, but also as to virulence. The clinical significance of differences in invasive potential has not yet been elucidated, but the fact that such differences can occur makes it of fundamental importance that they be documented and studied.

Earlier reports, in the middle 1960's, showed a wide spread of virulence over a series of strains collected from burn patients. However, the identity of epidemic producing strains continues to vary and prolonged use of topical therapy in control of wound sepsis has made further study of this epidemiologic phenomenon desirable. Selection of strains has been based primarily on their exhibiting virulent attributes. They were from blood culture, wound biopsy, or from tissues post mortem, including lung and liver isolates as well as wound strains. Table 1 summarizes the range of virulence demonstrated. There was a complete range of virulence: 4 strains out of 70 were completely non-virulent; 9 more were weakly virulent; none killed more than 7% of the animals seeded. There were 2 types in each of these categories. In the intermediate range, which included up to 60% mortality, 20 strains which included 4 phage types were found. Six phage types and 37 strains exhibited high virulence. Each lethality value was determined on not less than 4 groups of animals, so that cumulative results confirmed the validity of the virulence level.

A unique degree of virulence associated with a specific phage type, A-71, has been observed in detail, in part because of its appearance as an epidemic strain associated with a high rate of Pseudomonas sepsis. When the strain reached epidemic proportions in 1973, retrospective review of the culture collection uncovered a strain of the same type recovered 8 years earlier, in 1965. It was at that time unique, and was not seen again for 8 years. It has been found since its epidemic outbreak but so seldom that it would not, except for its history, have been observed further. Table 2 shows the persistence

Table 1. Virulence of 70 Strains of *Ps. aeruginosa*
Representing 14 Phage Types Recovered from Burn
Patients, 1973-1975

<u>Types and % Died Out of Total Rats Seeded</u>							
Non-Virulent (No deaths)	Low Virulence (1 - 19%)		Moderate Virulence (20 - 59%)		High Virulence (60 - 100%)		
NT-16	NT-3	2.3	NT-4	25	NT-5	82.7	
NT-A	NT-A ¹	6.4	NT-18	58.4	NT-17	94.5	
			NT-B	48.1	NT-2	67.3	
			F-21	29.2	D-5	92.4	
					C-26	84.3	
					A-71	99.2	
4 Strains	9 Strains		20 Strains		37 Strains		
2 Types	2 Types		4 Types		6 Types		

of virulence in this phage type. It is unique in our experience with hundreds of strains of *Ps. aeruginosa* in that the strain is so uncommon, (except in epidemics) that it could not have persisted in the burn ward, but has been recovered as a new entry into this population. Nonetheless, this strain is specifically associated with a uniquely high virulence for the burned rat. This has not been the case with other virulent epidemic strains, when they have been assessed for virulence. As a model for studying the constancy of virulence, strains from a burn patient who underwent at least 2 separate septicemic episodes during his illness were studied. Their behavior is summarized in Table 3. This patient was invaded first by type F-21. Three successive isolates, identical by biochemical and phage criteria, were assessed. Significant differences in lethality occurred: 1 isolate was lethal for only 15% of untreated rats, another for 25% and the third for 44%. The progression of response to treatment paralleled the rise in virulence, from 100% effectiveness with the least virulent, to 88% effectiveness in the most virulent strain.

A subsequent and more severe episode of *Pseudomonas sepsis* occurred a week later in this patient's course. This time the infecting strain was a different type, NT-2. Virulence varied from 42% to 89% in strains from the blood; 3 wound strains were all of the same level of virulence, and were probably identical. Thus, within one patient, 2 separate strains yielded isolates with 5 different degrees of virulence. Response to therapy was consistent with the concept that the more virulent the strain was, the higher

**Table 2. Virulence of Tested Animals of 19 Strains of
Ps. aeruginosa, Type A-71, Recovered from 18 Burned
Patients Between 1965 and 1975**

Year	No. of Patients	No. Strains A-71 Tested	Virulence Died/Total, %	Average Time to Death (Days)
1965	1	1	30/30 100	6.7
1973	15	16	449/452 99.3	5.56
1974	1	1	10/11 90.1	7.5
1975	1	1	10/10 100	6.4

**Table 3. Animal Model Virulence and Response to
Therapy of Ps. aeruginosa from a Patient with Lethal Burn Wound Sepsis**

Strain	Source	Type	No. of Tests	Mortality and Treatment Response			
				Sulfamylon	% Died	No Treatment Controls	% Died
9-25-36	Biopsy	F-21	4	0/20	0	3/20	15
9-27-13	Biopsy	F-21	5	3/25	12	11/25	44
9-28-9	Wound Swab	F-21	4	1/20	5	5/20	25
10-3-10	Biopsy	NT-2	4	7/26	27	16/26	62
10-3-11	Biopsy	NT-2	5	8/25	32	17/25	68
10-3-12	Wound Swab	NT-2	5	5/26	19	16/26	62
10-4-22	Blood	NT-2	4	8/20	40	17/19	89
10-5-15	Blood	NT-2	4	0/20	0	8/19	42

Strain Sources: Type F-21: Wounds: 25-28 Sept.
 NT-2: Wounds: 3 Oct
 NT-2: Blood 4-5 Oct

Control Strains: 8-28-3: Treat: 0-20% mortality
 No Treat.: 90-100% mortality

the proportion of treatment failures. This has not always been the case, in other experiments, high and low virulent strains were each completely susceptible to topical Sulfamylon treatment.

The experimental behavior of Ps. aeruginosa strains recovered from burn patients is not simply a case of virulent versus non-virulent organisms. Some strains are completely virulent, with no non-virulent variants observed. Other strains vary widely in virulence even with the most meticulous retrieval and storage technics. Further, virulent and non-virulent strains vary in their response to topical therapy. The assessment of new drugs and formulations can be made simple by using a "standard" strain that behaves in a consistent - i.e., desired manner. But the natural history of *Pseudomonas* burn wound infection is far more complex, and involves strain-related differences that must be taken into account if valid guides to clinically useful therapeutic agents are to be achieved.

EFFECT OF ADDITION OF TWEEN-80 TO INOCULUM ON VIRULENCE AND RESPONSE TO THERAPY

Variations occur in response of burned rats to seeding with a given strain of Ps. aeruginosa. These fluctuations can interfere with interpretation of the nature of an infecting strain, and with evaluation of a therapeutic regimen. One possibility, frequently discussed as a possible factor, is the degree of dispersal of cells of the inoculum in growth; Ps. aeruginosa may form non-homogeneous suspensions of bacteria when they are grown in broth. A means of achieving more uniform dispersal, and of improving the wetting qualities of the culture on the burn wound, is to grow the culture in broth to which a small amount of non-toxic wetting agent has been added. This experiment was conducted with 2 challenge strains, 8-28-3 and 12-4-4, to determine whether or not demonstrable differences in lethality or in response to therapy could be demonstrated.

Five different strains, varying in lethality and in response to topical Sulfamylon therapy were used. Strain 8-28-3 is highly virulent; strain 12-4-4 is virulent but permits more survival; and A-71 represents a most lethal treatment refractory strain, with A-71-2 virulent but somewhat treatment responsive, and A-71-3 low in virulence yet treatment refractory. Table 4 summarizes the results.

There was no significant increase in lethality of strains in animals not treated, between the effect of the Tween-80 cultures and the Trypticase Soy Broth (TSB) cultures. Among treated animals, there was a slight increase in lethality despite treatment, although it was not statistically significant. Among the 7 or 9 experiments, with a total of 17 sets run, 11 of 88 of the Tween-80 broth animals died, while in the same set the TSB inoculum resulted in death of 5 out of 90 animals. It may be concluded that the wetting agent does not enhance the virulence of the challenge strain, nor does it significantly alter the response to therapy. There may be slightly more animals which die when treated following Tween-80 inoculum than with the TBS inoculum.

Table 4. Effect of 0.1% Tween-80 in Inoculum on Virulence and Response to Therapy of *Pseudomonas aeruginosa* in Burned Rats

Exp. No.	Strain	Substrate	Treated with Sulfamylon 10%		Controls	
			Died/Total	Survival* Time	Died/Total	Survival Time
1	8-28-3	Tween-80 TSB**	4/15 1/15	18.27 + 6.2 22.00	15/15 14/15	6.33 + 2.47 6.93 + 4.3
2	8-28-3	Tween-80 TSB	1/8 0/8	19.88 + 6.01 22.00	6/7 7/7	9.43 + 6.08 10.14 + 2.85
3	12-4-4	Tween-80 TSB	1/7 0/7	20.28 + 4.53 22.00	7/7 6/6	9.86 + 1.85 8.33 + 1.20
4	4-24-14 (A-71)	Tween-80 TSB	14/15 14/15	8.27 + 4.73 6.87 + 5.3	14/15 13/14	7.60 + 4.12 7.36 + 4.75
5	4-28-12 (A-71-3)	Tween-80 TSB	2/15 0/14	20.53 + 3.9 22.00	3/15 1/13	20.20 + 3.8 21.46 + 1.9
6	4-28-13 (A-71-3)	Tween-80 TSB	1/15 1/14	21.47 + 2.07 21.50 + 1.87	0/3 1/13	22.00 20.62 + 5.0
7	5-15-2 (A-71-2)	Tween-80 TSB	2/15 0/14	20.80 + 3.2 22.00	13/14 14/14	10.36 + 3.75 10.72 + 2.55
8	5-28-16 (A-71-2)	Tween TSB	8/14 14/15	14.08 + 6.95 9.73 + 4.86	13/13 13/14	7.08 + 2.8 9.43 + 4.4
9	3-28-3	Tween-80 TSB	0/13 3/14	22.00 19.86 + 4.88	13/13 13/14	7.00 + 1.73 11.07 + 5.35

* Survival time: Terminated on day 22; Survival = 22.0 days

** TSB: Trypticase Soy Broth

Since the wetting agent makes it noticeably easier to apply the 1.0 ml inoculum to the freshly burned rat, this modification has been introduced into the routine rat challenge model.

PIRBENICILLIN, A NEW SEMI-SYNTHETIC PENICILLIN TRIAL IN CONTROL OF EXPERIMENTAL PSEUDOMONAS BURN WOUND SEPSIS

Perbenicillin, a new injectable semi-synthetic penicillin has a broad spectrum of antibacterial activity. Its activity is comparable to that of carbenicillin although it is reported to have enhanced activity against fecal streptococci, *Pseudomonas* and *Enterobacteriaceae* that do not produce beta-lactamase. In view of the long standing need for a more effective systemic antimicrobial agent which can control *Ps. aeruginosa* invasive infection in burns, and in the hope that this agent might be of value in controlling other opportunistic invading organisms in burns, it was tested in the invasive *Pseudomonas* burn wound sepsis model, using 200 gm rats with a 20% scald burn, seeded promptly with 10^8 cells of an 18 hour broth culture. *In vitro* tests on *Ps. aeruginosa* were carried out using the MIC technic standardized in this laboratory.

The sensitivity to antibiotics of 3 bacterial species of major importance in burn wound infections are summarized in Table 5. Staphylococci were relatively susceptible to the antibiotic, *Ps. aeruginosa* were moderately sensitive, and *Klebsiella pneumoniae* were resistant. The remaining species of *Enterobacteriaceae* were tested in small numbers; their behavior is an indication of sensitivity but numbers were few.

Table 5. Sensitivity of Bacterial Species Prominent in Current Burn Infections to Pirbenicillin

Species	No. Inhibited and Concentration of Pirbenicillin (mcg/ml)									
	> 50	50	25	12.5	6.25	3.12	1.5	0.78	0.39	< 0.39
<i>S. aureus</i>	0	0	0	0	5	15	5	1	0	0
<i>K. pneumoniae</i>	28	2	2	0	0	0	0	0	0	0
<i>Ps. aeruginosa</i>	1	1	5	3	0	1	0	0	0	0
<i>E. coli</i>	2	0	0	0	0	0	0	0	0	0
<i>S. marcescens</i>	2	0	0	0	1	0	0	0	0	0

Graphic representation of cumulative sensitivity gives a more informative picture of the sensitivity status. These data are shown in figure 1. The results

CUMULATIVE SENSITIVITY OF KLEBSIELLA PNEUMONIAE, STAPHYLOCOCCUS
AUREUS AND PSEUDOMONAS AERUGINOSA STRAINS TO PIRBENICILLIN

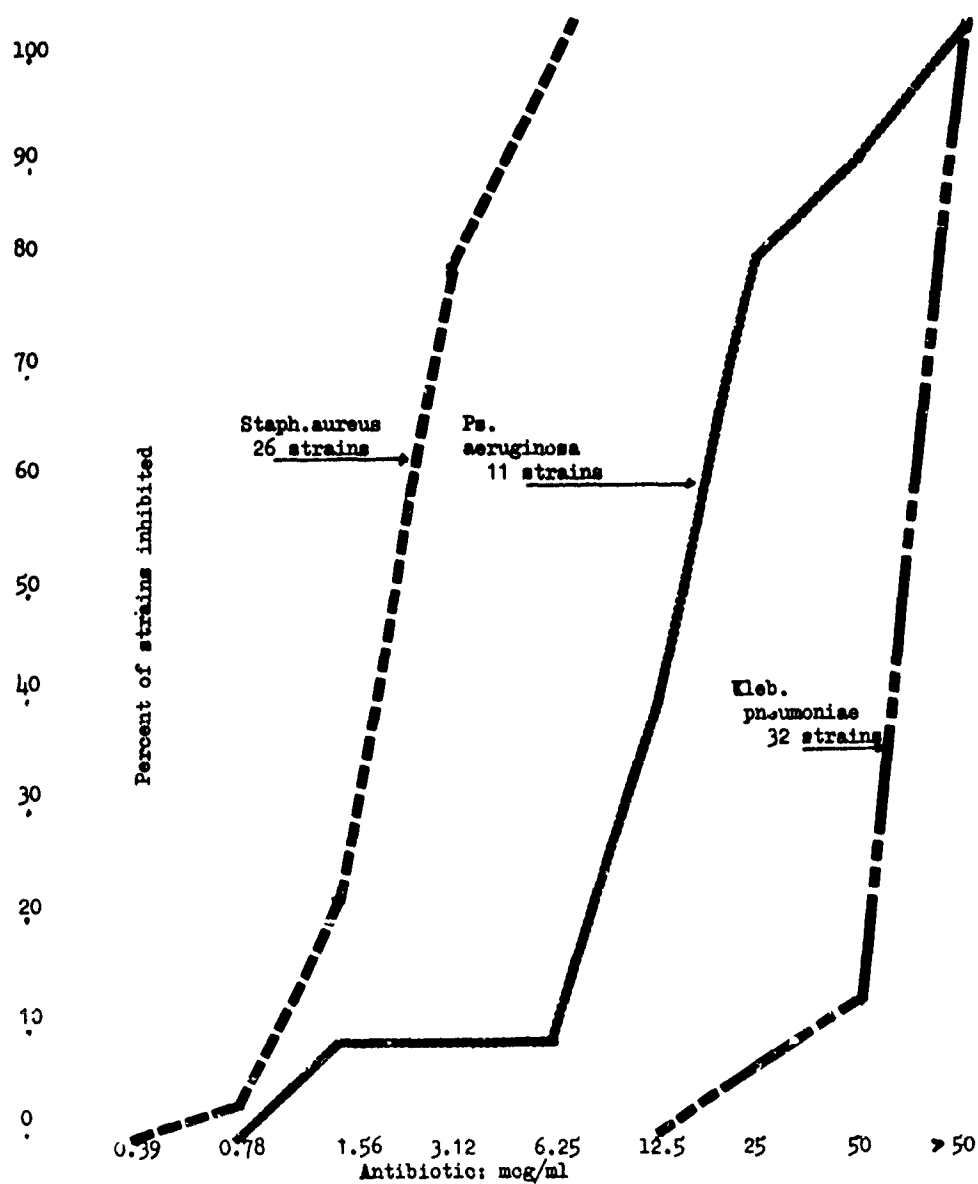


Figure 1.

make more emphatic the inherent resistance of *Klebsiella* strains and the intermediate status of *Pseudomonas*. Only *Staphylococcus aureus* was sensitive to Pirbenicillin.

The use of Pirbenicillin in control of experimental burn wound sepsis was based on the encouraging results in clinical trials reported in communications from research laboratories of the manufacturer, Pfizer, Inc. Pharmacologic data indicated that the drug was tolerated in doses up to 250 mg/kg, via subcutaneous administration, in rats. After preliminary trials, dosages of 250 mg/kg and of 125 mg/kg, intraperitoneally on a 1 X/day basis, were selected. Dosage schedules of 5 and 10 days were carried out in groups of 5 or 6 rats each. Treatment was begun 24 hours after seeding of the rats. Animals were observed for 21 days after burning and seeding. The criterion of effectiveness was survival. Challenge strains included 12-4-4, 8-28-3 and VA-134, to cover a spectrum of virulence and invasiveness.

Results are summarized in Table 6. The 3 challenge strains varied slightly in their response to Pirbenicillin, but none of the results was encouraging to a significant extent. With strain 12-4-4, the maximum dose presented a 70% mortality with a strain which, untreated, killed 85.6% of the animals. Strain 8-28-3 was more virulent in the untreated control, but it showed the most encouraging results *in vivo*: maximum dose for maximum time showed a 29.4% mortality; when the 250 mg/kg/day dose was run for 5 instead of 10 days, mortality rose to 64.7%. The reduced dose of 125 mg/kg/day actually showed the best survival of all; it was the same for the 5 day as for the 10 day interval. The most virulent challenge strain, VA-134, showed a slight response to the maximum dose, and virtually no effect with the minimum dose.

Table 6. Pirbenicillin in Prophylaxis of Burn Wound Infection in Rats Seeded with *Pseudomonas aeruginosa*

Dosage mg/kg	Treatment Period	No. of Tests	Strain and Outcome - Died/Total (% Mortality)		
			<u>12-4-4</u>	<u>8-28-3</u>	<u>VA-134</u>
250	10 days	3	12/17 (70.5)	5/17 (29.4)	10/17 (58.8)
	5 days	3	12/17 (70.5)	11/17 (64.7)	13/17 (76.4)
125	10 days	3	11/17 (64.7)	9/17 (52.9)	12/17 (70.5)
	5 days	3	12/17 (70.5)	8/17 (47.0)	16/17 (94.1)
Control			13/15 (85.6)	13/13 (100)	16/16 (100)

DISCUSSION

The results of animal trials of Pirbenicillin in control of Ps. aeruginosa burn wound sepsis were not encouraging. The drug had a slight effect in increasing survival, but it was not as effective as Carbenicillin in similar circumstances and far less effective than Ticarcillin which was shown to have a high potential for controlling invasive *Pseudomonas* infection in the seeded burned rat. On the basis of this particular model and challenge system, there is little enthusiasm for a clinical trial of Pirbenicillin.

PRESENTATIONS

Lindberg RB: Hospital Epidemics of Drug-Resistant Pseudomonas aeruginosa Infections. Am. Soc. Microbiol., New York, 28 May 1975.

PUBLICATIONS:

Lindberg RB, Latta RL, Pruitt BA, Jr: Transfer and Control of Hospital Epidemics of Drug-Resistant Pseudomonas aeruginosa (Abst). Am. Soc. Microbiol. 1975, p. 40 (C-83).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY ^a	4. KIND OF SUMMARY ^a	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. ORIGIN INSTN ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS ^a	10. LEVEL OF SUM ^a
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10 NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	1102A	3A161102B71R	01	219			
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) The Role of Fungi in Burn Wound Infection: Observations on Biopsy and Autopsy Tissues From Seriously Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY ^a		16. PERFORMANCE METHOD	
66 02		76 09		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. FUNDS (In thousands)	
a. DATES/EFFECTIVE:		EXPIRATION:		PRECEDING		FISCAL YEAR	
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e. AMOUNT:				CURRENT		7	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
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RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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22. GENERAL USE				23. ASSOCIATE INVESTIGATORS			
FOREIGN INTELLIGENCE NOT CONSIDERED				NAME: Anthony A. Contreras, MS			
				NAME: DA			
24. REVERSALS (Precede EACH with Security Classification Code)							
(U) Fungi; (U) Mucor; (U) Rhizopus; (U) Fusarium; (U) Burns; (U) Phycomycosis; (U) Humans							
25. TECHNICAL OBJECTIVE, 26. APPROACH, 27. PROGRAM (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
23. (U) To determine species of fungi in burn patients and assess the significance of these opportunistic invaders in producing fungal burn wound sepsis, respiratory tract involvement and other infectious processes peculiar to infection in burned soldiers.							
24. (U) Cultivation of biopsy and autopsy specimens under optimal conditions for recovery of fungi and yeasts.							
25. (U) 75 07 - 76 09 The occurrence of fungal colonization diminished further in FY 1976. Of 135 biopsies on 63 patients, 5 patients yielded 6 fungal cultures, while 11 patients yielded 13 strains of Candida sp. In autopsies, 38.8% of patients yielded Candida sp.; only 23.6% of autopsies yielded fungi in culture. <u>Fusarium</u> and <u>Aspergillus</u> were genera more frequently recovered; 9 genera were recovered and 38 strains were isolated. No phycomycetes were found. Fungal sepsis remains a possibility but in 1975 it was relatively uncommon.							

^a Available to contractors upon sponsor's approval.

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PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1490A 1 NOV 63 AND 1496 1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE - SURGERY

**REPORT TITLE: THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS
ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED
SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 June 1976

Investigators:

Robert B. Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES-- SURGERY

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ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED
SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 June 1976

Investigators: Robert B. Lindberg, PhD

Reports Control Symbol MEDJH-288(R1)

Autopsy and biopsy tissues, including wound, lung, spleen, liver and burn wound in autopsy, are routinely cultured for fungi. Fungal sepsis has not been a serious clinical problem since 1973, but fungal colonization still occurs. The recovery rate of yeasts and fungi fell from 71 - 75% in recent years to 52% in 1975. Predominant genus was Fusarium. Phycomycetes, the most dangerous fungi when invasive infection occurs, were not recovered in 1975.

Fungi
Mucor
Rhizopus
Fusarium
Burns
Phycomycosis
Humans

THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

Fungal infection in burn wounds has followed an unpredictable course. Individual institutions have seen such invasive infections increase to a point at which they were a serious problem in morbidity and mortality. The incidence may then regress, with no valid basis in changes in wound management. In the Institute of Surgical Research burn wound invasion by fungi has become rare, although 4 to 5 years ago it was a problem involving 5 to 10 patients per year, in a pathological process that was either uncontrollable or controlled with ablation of involved tissues when such surgical intervention was possible.

Fungi in Biopsy Specimens

Biopsies were performed on 63 patients in 1975, and 135 specimens were collected. Results of cultures of tissues on Sabourauds agar are set forth in Table 1. Comparative results for 1972 - 1974 are set down. It was obvious that far fewer biopsies were collected in 1975 than had been the case in preceding years and the scale of recovery of fungi had correspondingly narrowed. Thirteen genera of fungi were recovered in 1972, 10 in 1973 and 7 in 1974; in 1975 4 genera were recovered from burn wounds. Had it not been for preceding years and the importance of the role played by fungi; the very procedure might have been discontinued. However, the pathologic evidence of a significant role being played by fungi is, on an overall view, too important to be ignored.

Fungi Recovered from Autopsy in Burned Patients

Fungi recovered from burn wounds and viscera (lung, liver and spleen) at autopsy are summarized in Table 2. There were 9 genera of fungi recovered, as always, these organisms were more common in tissues at autopsy. Presumably one reason for this discrepancy is the ease with which opportunistic microorganisms proliferate in tissues of the immunologically impaired host. There were 9 genera of fungi, including the Candida spp. recovered. The number of isolates was small, but Fusarium spp. were the predominant genus, as they had been in the preceding two years. Aspergillus spp. were the other major fungal form recovered. No genera with a pedigree of ability to cause invasive burn wound infection were recovered.

The nature of the decrease in frequency of recovery of fungi in burned patients in the ISR is most clearly shown in a comparison of recoveries made since 1971. These totals are shown in Table 3.

It was obvious that fewer positive fungal cultures appeared in 1975 than in recent years. In the interval between 1971 and 1975, the fraction of patients cultured who yielded fungi or yeasts from tissues ranged from

Table 1. Biopsy Specimens Positive for Fungi
and Yeast - 1972 - 1975

Genus	No. Patients Positive				No. Strains Recovered			
	1972	1973	1974	1975	1972	1973	1974	1975
Aspergillus	8	8	8	1	11	17	5	2
Cephalosporium	5	4	5	1	15	5	5	1
Fusarium	19	12	11	2	33	23	17	2
Sepedonium	0	1	0	0	0	1	0	0
Curvularium	3	2	1	0	3	2	3	0
Scopulariopsis	11	0	0	0	11	00	0	0
Alternaria	3	2	3	1	7	2	3	1
Diplosporium	1	0	0	0	1	0	0	0
Penicillium	1	1	3	0	1	1	3	0
Helminthosporium	0	4	2	0	0	9	2	0
Rhizopus	2	2	0	0	3	2	0	0
Mucor	2	2	0	0	2	2	0	0
Stemphyllium	1	0	0	0	1	0	0	0
Syncephalastrum	1	0	0	0	1	0	0	0
Geotrichum	1	0	2	0	1	0	4	0
Candida sp.	28	57	54	11	46	141	144	15
No. Patients Cultured			135	63				

Table 2. Genera of Fungi Recovered from Viscera
(Liver, Lung, Spleen) and Burn Wound at Autopsy
1975

Genus	Wounds		Viscera	
	Patients	Positive Total Isolates	Patient Positive Total Isolates	Total Isolates
Aspergillus	3	3	9	12
Penicillium	1	1	0	0
Cephalosporium	1	1	2	2
Fusarium	5	5	3	6
Scopulariopsis	1	2	0	0
Curvularium	2	3	1	1
Alternaria	0	0	3	4
Sepedonium	1	1	2	2
Mycelia sterilia	0	0	1	1
Candida	12	21	23	48

Table 3. Fungi Recovered from Autopsy Tissues, 1971-1975

Genus	Number of Patients Positive at Autopsy									
	Burned Wound					Viscera				
	1971	1972	1973	1974	1975	1971	1972	1973	1974	1975
Mucor	3	0	3	3	0	0	0	1	1	0
Rhizopus	1	1	2	1	0	1	1	0	0	0
Absidia	1	1	0	0	0	0	0	0	0	0
Aspergillus	6	11	19	4	3	3	2	5	2	9
Penicillium	8	1	0	1	1	1	2	0	5	0
Paeciliomyces	0	0	0	0	0	1	2	0	0	0
Alternaria	2	3	6	1	0	0	0	0	2	3
Cephalosporium	2	4	3	4	1	0	4	4	8	2
Fusarium	9	30	25	12	5	7	20	19	8	3
Helminthosporium	4	1	4	4	0	0	0	2	1	0
Nigrospora	9	0	0	0	0	3	0	0	0	0
Scopulariopsis	5	3	1	2	1	1	2	0	2	0
Sepedonium	1	5	5	1	1	1	4	3	1	2
Diplosporium	1	2	0	0	0	0	0	0	0	0
Geotrichum	0	0	3	0	0	2	0	0	1	0
Fonsecaea	2	0	0	0	0	2	0	0	0	0
Curvularia	0	5	1	0	2	0	0	0	0	1
Microsporium	0	1	0	0	0	0	0	0	0	0
Cladosporium	0	0	1	0	0	0	0	3	0	0
Candida	13	32	67	41	12	11	25	50	42	23
Number patients positive	46	65	80	67	39					
Number patients cultured	61	89	91	80	74					
Number genera present	17	16	14	15	9					

73% to 83%. In 1975, 52% of the patients cultured yielded yeasts or fungi.

The absence of Phycomycetes in biopsy and autopsy in 1975 was significant since this group includes the Mucor, Rhizopus and *Obsidia* genera. These can and have caused invasive burn wound sepsis with very lethal consequences.

The trend toward lessened incidence of invasive fungal infection of burn wounds was apparent in 1973, and since that time this disease has virtually disappeared in the ISR. The low recovery rate in both autopsy and biopsy in 1975 was gratifying, but there has been no specific step that can be regarded as controlling fungi. Continued monitoring is especially pertinent since interpretation of clinical disease may be aided very much by adequate comprehensive autopsy and biopsy culturing.

PRESENTATION AND/OR PUBLICATION

None

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				DA OF 6398	76 10 01	DD-DR&E(AR)436	
3. DATE PREV SUMMARY	4. REV OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISSEM INSTRN	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF DOW
75 07 01	H. TERM	U	U	NA	NL	<input type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		61102A		3A161102B71R		01	
B. CONTRIBUTING						319	
C. CONTRIBUTING							
11. TITLE (Provide with Security Classification Code) (U) Non-Fermentative Gram-Negative Bacilli in Burned Soldiers New Potential Opportunistic Pathogens (44)							
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10. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
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RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish D&A if U.S. Academic Institution)			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
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				NAME: Virginia E. English, MS			
				NAME:		DA	
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23. (U) Most infections in severely burned soldiers are due to opportunistic flora and there have been several species recognized as having major pathogenic potential only after they had been found in cases of sepsis in burns. Detection of potential pathogens capable of setting up nosocomial infection is merited as enlarging knowledge regarding wound infection potentialities in injured soldiers.							
24. (U) Special culture technics and substrates are used as indicated by biology of detected groups.							
25. (U) 75 07 - 76 09 Significant numbers of 9 species, 8 of which were oxidative and one fermentative, were recovered from autopsy and biopsy specimens. High concentration in some quantitative tissue cultures ruled out casual contamination of a source. Potential burn wound pathogens have thus been exposed.							

* Available to contractors upon originator's approval

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TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

**REPORT TITLE: NON-FERMENTATIVE GRAM-NEGATIVE BACILLI IN BURNED
SOLDIERS: NEW POTENTIAL OPPORTUNISTIC PATHOGENS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Virginia C. English, MA
Robert B. Lindberg, PhD**

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: NON-FERMENTATIVE GRAM-NEGATIVE BACILLI IN BURNED SOLDIERS: NEW POTENTIAL OPPORTUNISTIC PATHOGENS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

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Oxidative, non-fermentative gram-negative bacteria have been found in significant numbers as predominant organisms in tissues at autopsy and/or biopsy of burned patients. These species are seldom recovered from clinical specimens; their relatively slower growth and fastidious character make overgrowth by Enterobacteriaceae a major complicating factor in their detection. Eighteen patients harbored one or more of 10 species, with Pseudomonas maltophilia, Pseudomonas putida, Acinetobacter calcoaceticus and Acinetobacter lwoffii as predominant forms. Their presence as predominant organisms at autopsy suggests that they may play a significant, if unrecognized, role in burn morbidity and mortality. As relatively new opportunistic invaders, they should be sought with more effort than is presently routinely made.

Burns
Oxidative gram-negative bacilli
Pseudomonas
Acinetobacter
Alcaligenes
Flavobacterium
Humans

NON-FERMENTATIVE GRAM-NEGATIVE BACILLI IN BURNED SOLDIERS NEW POTENTIAL OPPORTUNISTIC PATHOGENS

From the first recognition of infectious disease as caused by bacteria, there has been a continually expanding recognition of pathogenic microorganisms. Specific diseases were associated with causative agents, and even in non-specific situations such as wound infections, emphasis was placed on aerobic cocci and Clostridia as the principal offenders. From pre-bacteriologic time, however, records point to one non-fermentative bacillus, B. pyocyaneus, now designated Pseudomonas aeruginosa. The blue-green pigment seen on wound dressings, which incidentally often prompted a favorable prognosis, was and still is caused by this ubiquitous organism, which is innocuous for healthy individuals but which can act as a lethal invasive pathogen in severe burns, debilitated individuals, or in immunologically compromised individuals. An increasing number of gram-negative bacilli other than classic Enterobacteriaceae species have been recognized in recent years as capable of opportunistic pathogenic activity. Many of these are oxidative rather than fermentative in their utilization of carbohydrate. Their pathogenic activity can involve generalized sepsis, especially in infants and children, but their primary interest in burn patients revolves around their role as opportunistic wound invaders.

METHODS

Routine diagnostic procedures on wound, blood, sputum and other body fluids do not exclude specific search for obscure species, but it has not been feasible to scrutinize exhaustively every clinical specimen. However, tissues from wound, lung, liver and spleen are cultured quantitatively in autopsies, as are biopsy specimens, and the highest dilution with discrete colonies is scrutinized in detail for unusual organisms. Colonization of burn wound, pneumonia or septicemia caused by organisms sequestered in spleen and liver could be revealed by such examination, and the extent of recovery of unusual species has validated this supposition. A significant number of oxidative organisms of species not previously observed in the Institute of Surgical Research patients have been recovered.

UNUSUAL GRAM-NEGATIVE BACILLI RECOVERED IN 1975

Unusual, mainly oxidative, non-fermentative gram-negative bacilli recovered from high dilution plates at autopsy are summarized in Table 1. There were 18 patients, out of 76 autopsies, in whom one or more of these unusual species were recovered. Thus, 23% of autopsies of burn deaths showed one or more of these species predominant in wound and/or viscera. This is not an inconsequential rate of colonization and potential infection, and makes continued search for these organisms a highly desirable procedure.

There were 10 species recovered. Their distribution by numbers of patients and by source is shown in Table 2. The predominant species was

Table 1. Uncommonly Isolated Gram-Negative Nonfermenting Organisms from Postmortem Tissues - 1975

Autopsy No.	Patient No.	Source	Organism
A-3-75	3	Spleen:	Acinetobacter lwoffii
A-4-75	5	Tissue Bx. # 4:	Acinetobacter lwoffii
A-5-75	240*	Spleen:	Pseudomonas maltophilia
A-6-75	15	LUL, Liver, Spleen, RLL: Pseudomonas maltophilia LUL, RUL, RLL, Spleen: Pseudomonas putida	
A-7-75	6	Liver:	Flavobacterium, Type II-B
A-9-75	30	Spleen: Moraxella osloensis; Acinetobacter calcoaceticus Tissue Bx. # 2 Acinetobacter lwoffii; Pseudomonas maltophilia Tissue Bx. # 8, Liver; LUL, RUL, Spleen, LLL: Pseudomonas maltophilia	
A-10-75	25	Liver :	Pseudomonas maltophilia
A-11-75	23	Spleen, RLL, Liver: Pseudomonas maltophilia LLL: Pseudomonas putida	
A-12-75	28	Spleen: Apyocyanogenic Pseudomonas aeruginosa	
A-28-75	46	Spleen: Apyocyanogenic Pseudomonas aeruginosa RLL: Achromobacter, biotype 3, Pseudomonas maltophilia LLL, RUL: Pseudomonas maltophilia	
A-29-75	RG	Liver:	Pseudomonas maltophilia
A-30-75	86	Liver: Acinetobacter calcoaceticus LLL, RLL: Pseudomonas maltophilia	
A-31-75	80	Spleen: Acinetobacter calcoaceticus Pseudomonas maltophilia	
A-38-75	109	Liver, LLL, Spleen: Achromobacter sp. RUL, LUL: Pseudomonas putida	
A-39-75	96	RLL, LUL, Spleen: Achromobacter sp.	
A-41-75	128	RUL: Achromobacter sp.	
A-45-75	137	Tissue Bx. # 4: Acinetobacter calcoaceticus	
A-34-75	85	RUL: Citrobacter diversus*	

* Organism recovered in 1975 from a patient admitted in 1974.

Pseudomonas maltophilia, found in 9 patients. Antemortem sepsis has been suggested as a plausible source of sequestration of bacteria in viscera. Acinetobacter lwoffii, formerly classified as Mima polymorpha, and Acinetobacter calcoaceticus, the former Herellea vaginicola, were similar in rate of occurrence. Pseudomonas putida, and 2 species of Achromobacter, were each recovered from 3 autopsies. The total recovery rate was similar to earlier studies of this material. The smaller totals for 1975 are due to a more limited series of examinations.

Table 2. Patients and Sites From Which Oxidative Organisms Recovered at Autopsy, ISR - 1975

Species	No. Patients Positive	Sites of Recovery			
		Wound	Spleen	Liver	Lung
A. lwoffii	3	2	1		
Ps. maltophilia	9	2	5	5	4
Ps. putida	3		1		2
Flavobact. II, B	1			1	
Moraxella osloensis	1			1	
A. calcoaceticus	4	1	2	1	
Apyocyaninogenic Ps. aeruginosa	2		2		
Achromobacter Type 3	3		2	1	3
Achromobacter sp	3		2		3
Citrobacter diversus	1				1

It was observed that patients in whom a non-fermenting, exotic organism was found were especially prone to harbor more than one species. Seven patients, out of the 18 positive for an oxidative species, harbored more than one species. This sequence is shown in Table 3. Ps. maltophilia, in parallel with its overall incidence, was found in 5 of the 7 multiple infections. Liver and lung were each positive in 5 patients, the spleen in 5, while only one patient with multiple organisms had a mixed culture in samples of the burn wound.

Antemortem isolations were less common than those recovered at autopsy, but there were 5 species recovered from clinical specimens. Their occurrence, in connection with postmortem recoveries, and the total of isolates of each species of oxidative microorganisms, are presented in Table 4. Apyocyaninogenic Ps. aeruginosa, Ps. putida, Ps. maltophilia and Ps. acidovorans were recovered antemortem, the first 3 were also conspicuous in autopsy material.

Table 3. Patients Whose Postmortem Tissues Presented Multiple Nonfermenting Organisms

Patient No.	Surface Biopsy	LIVER	SPLEEN	LUNG
15		P.maltophilia	P.maltophilia	P.maltophilia P.putida
30	A. lwoffii P. maltophilia	P. maltophilia	M. osloensis A. calcoaceticus	P. maltophilia
23.		P. maltophilia	P. maltophilia	P. maltophilia P. putida
46			Apyocyanogenic P. aeruginosa	Achromobacter
80		A. calcoaceticus		P. maltophilia
86		A. calcoaceticus P. maltophilia		
109		Achromobacter sp.	Achromobacter sp.	Achromobacter P. putida

Table 4. Incidence of Nonfermenting Organisms
Infrequently Recovered from Patients

ORGANISM	Number of patients	Number of Isolates	Antemortem Sources	POSTMORTEM SOURCES			
				Surface	spleen	liver	lung
<i>P. aeruginosa</i> , <i>Apyocyanogenic</i>	4	5	1 - Bx. L. arm 1 - Iukens	1	2	2	
<i>P. putida</i>	4	8	1 - Foley tip		1		6
<i>P. maltophilia</i>	10	24	1 - Blood	1	5	4	14
<i>P. acidovorans</i>	1	1	1 - urine				
<i>A. lwoffii</i>	3	3		2	1		
<i>Flavobacterium</i> , GP II-8	1	1					1
<i>M. osloensis</i>	1	1				1	
<i>A. calcoaceticus</i>	4	4		1	2	1	
<i>Achromobacter</i> sp.	4	8			2	1	4

The burn injuries incurred by the patients from whom oxidative organisms were recovered were relatively severe. The total burn and extent of third degree burn are shown in Table 5. Burn extent ranged from 46.5% to 93% of body surface. Five out of 8 patients had burns of over 60% total body surface. Sepsis in such injuries is not unusual. However, there was no unique quality about the injuries that would set them aside from injuries with more typical fermentative flora.

The strains of 3 species of *Pseudomonas* other than *aeruginosa* were examined for their sensitivity to Sulfamylon, since each of these had been recovered from patients whose wounds merited topical treatment to control invasive infection. Results are shown in Table 6. The strains were inhibited by 0.078% concentration.

Since a highly refined phage typing system for *Ps. aeruginosa* has been developed and serves to monitor this organism, a sample of strains of *Ps. putida*, *Ps. maltophilia* and *Ps. pseudoalcaligenes*, were tested with all 18 of the typing phages. No strains reacted at the routine test dilution, but 8 out of 10 of the *Ps. maltophilia* reacted with phage M-6 when it was used undiluted. The other 3 strains of 2 species did not type. It is of interest to note that *Pseudomonas fluorescens* strains, a species closely related to *Ps. aeruginosa*, were found to be susceptible, in a small proportion of isolates, to phage 44. Cross-species susceptibility in the genus *Pseudomonas* suggests a common cell structure that also makes pathogenic involvement with this genus more plausible.

The distribution of oxidative organisms was not typical of major epidemic pattern of spread, but the periods of positive recoveries came in clusters. Some episodes chronologically suggestive of patient-to-patient transmission occurred. The strains are slow growing and readily obscured by Enterobacteriaceae in pure culture, and their detection is probably far from adequate in laboratories not specifically geared to search for them. All of the autopsy and biopsy specimens were recovered as predominant organisms in tissues; this prompts the strong supposition that they played a significant role in the infection. They were undoubtedly present in mixed flora on the burn wound, and routine cultural procedures failed to detect them.

The history of recognition of opportunistic invaders in wounds has been a succession of situations in which the opportunist was initially dismissed as a contaminant and not of clinical significance. *Ps. aeruginosa* was at one time thus regarded, as were the various species of Enterobacteriaceae which are now recognized as major causes of sepsis in burn wound infections. The relatively obscure group of oxidative forms thus far accumulated in burns is not now a recognizable clinical problem, but their presence in large numbers in wounds and viscera at autopsy indicates that they can play a pathogenic role. *Ps. maltophilia* in particular merits closer observation. Monitoring such exotic species is required if a true picture of burn wound pathogenesis is to be obtained.

PRESENTATION

Table 5. Survey of Pseudomonas Maltophilia Recovered From ISR Patients

Total Patients: 10

Total Isolates: 24

ANTEMORTEM ISOLATE: 1

Patient No.: 133
Percent Burn: 37%
Percent of 3: 1%
Source: Blood.

POSTMORTEM ISOLATES: 23

Patient No.	Percent Burn	Percent of 3	Source
240*	67.0	18.5	Spleen
RG	63.0	14.5	Liver
15	77.5	53.0	LUL, Liver, Spleen, RLL
23	65.5	40.5	Spleen, RLL, Liver
25	62.5	3.0	Liver
30	51.0	31.5	A # 2, A # 8, Liver, Spleen, LLL, LUL, RUL
46	46.5	3.0	LLL, RLL, RUL
80	54.5	11.0	Spleen
86	93.0	62.5	LLL, RLL

* 1974 admission; culture received 1975.

Table 6. Special Studies on Pseudomonas Species
Other Than Aeruginosa

Patient No.	Sensitivity to Sulfamylon*	Reaction to P. aeruginosa Bacteriophage system**
<u>P. MALTOPHILIA:</u>		
30	0.078 ***	P-1 ****
15	-	P-1
23	-	P-1
28	0.078	P-1
80	0.078	P-1
RG	0.078	P-1
183	0.156 (3 isolates)	P-1
128	0.312	NT
46	0.078	P-1
241	0.078	NT
<u>P. PUTIDA:</u>		
23	0.156	NT
15	0.078 (2 isolates)	NT
<u>P. PSEUDOALCALIGENES:</u>		
241	0.039	NT

* Sensitivity procedure same as than for P. aeruginosa

** Typing system used which is presently used for typing P. aeruginosa

*** Inhibiting concentration expressed in gms/100 ml.

**** These reactions were obtained using concentrated phage;
Code P-1 denotes a single reaction with phage M-6.

English, VC. Isolation of Vibrio alginolyticus from wounds and blood of a burned patient. Presented at National meeting Am Soc for Med Technology, Chicago, Ill. 23 June 76

PUBLICATIONS

English VC, Lindberg RB: Isolation of Vibrio alginolyticus from wounds and blood of a burn patient. Am J Med Tech (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ⁶	2. DATE OF SUMMARY ⁷	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ⁸	6. WORK SECURITY ⁹	7. REGRADING ¹⁰	8A. ORG'S INSTR ¹¹	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO / CODES ¹²	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61102A	3A161102B71R		01	165		
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19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
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				NAME: Arthur D. Mason, Jr, MD			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Protein; (U) Burn; (U) Trauma; (U) Turnover; (U) Rats; (U) Albumin; (U) Edema							
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23. (U) To determine the cause of the dysproteinemia observed following burn injury and to determine if the more marked dysproteinemia seen in the presence of infection of the burn wound is an effect caused by some action of the bacteria. It is hoped that this will aid in understanding similar changes which are observed in burned soldiers.							
24. (U) Incorporation of (2- ¹⁴ C) glycine into the serum proteins of groups of control, burned, burned-infected, and treated burned-infected rats has been measured. Body albumin distribution was determined by radioimmunoassay using extracts of tissues obtained on the sixth day postburn. Measurements of water content of skin during the early postburn period are being made. Exchange rates of albumin between the intravascular and extravascular compartments will be measured.							
25. (U) 75 07 - 76 09 Groups of burned rats (20% body surface burn) untreated, or treated one hour postburn by subcutaneous injection of hyaluronidase or saline into the burn wound area all had lowered plasma volumes at 6 hours postburn. When provided with food and water ad lib, but given no further treatment, the plasma volumes of all groups had returned to normal at 24 hours and were greater than normal at 48 hours postburn. The water content of the burned skin was elevated at 6 hours and was similar for all groups. The water content of the burned skin remained elevated at 24 and 48 hours postburn but was consistently lower in the skin of rats treated with hyaluronidase than in the skin of the saline treated rats or of those which received no treatment.							

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

**REPORT TITLE: STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN
BURNED TROOPS - USE OF AN ANIMAL MODEL**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Wanda L. Brown, MS
Eleanor G. Bowler, PhM
Arthur D. Mason, Jr., MD**

Report Control Symbol MEDDH-288 (R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT T E: STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN
BURNED TROOPS - USE OF AN ANIMAL MODEL

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in report: 1 July 1975 - 30 September 1976

Investigators: Wanda L. Brown, MS
Eleanor G. Bowler, PhM
Arthur D. Mason, Jr., MD

Report Control Symbol MEDDH-288(R1)

The carcass and the unburned skin of burned-infected rats had reduced levels of albumin. This was in contrast to the tissues of burned rats without infection in which the albumin content was essentially normal. The albumin content of the burned skin was elevated in both groups of rats.

Groups of burned rats (20% body surface burn) which were untreated, or were treated at one hour postburn by subcutaneous injection of hyaluronidase or saline into the burn wound, all had lowered plasma volumes at 6 hours postburn. When the rats were given free access to food and water, but no further treatment, the plasma volumes of all rats returned to normal at 24 hours and were greater than normal at 48 hours postburn.

Water content of the burned skin of these rats were elevated at 6 hours postburn but was similar for all groups. The water content of the burned skin of all of the rats was still elevated at 24 and 48 hours postburn, but it was consistently lower in the burned skin of the rats which had been injected with hyaluronidase.

Protein
Burn
Trauma
Turnover
Rats
Albumin
Edema

STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN BURNED TROOPS - USE OF AN ANIMAL MODEL

From the results of earlier studies in which we used the incorporation of (2-¹⁴C)glycine into serum proteins of burned and burned-infected rats to estimate synthesis on the 6th day postburn, we concluded that decreased synthesis was not the cause of the prolonged hypoalbuminemia typically observed following burn injury. Measurements of tissue albumin content demonstrated that the tissue albumin pool size of burned rats was greater than normal, and that the elevated albumin content of the burn wound accounted for the total increase in the pool size. Details of the procedures used and the results have been reported.

Since that report we have measured the albumin content of the individual tissues of a group of burned-infected rats. The mean albumin contents (mg/gm tissue) in the 3 groups of rats are shown in Table 1. In contrast to the burned rats which had essentially normal levels of albumin in carcass and in unburned skin, the burned-infected rats had reduced albumin content in those tissues. Despite this, the burned-infected wound area contained 3.5 times the amount of albumin in the unburned skin of the burned or control rats and contained 6 times the amount present in the unburned skin of burned-infected rats.

Table 1. Plasma and Tissue Albumin Content of Rats

	Mg Albumin/gm Tissue (wet wt)		
	Control N=3	Burned N=5	Burned-Infected N=3
Carcass	2.14 ± 0.10	2.02 ± 0.10	1.51 ± 0.13
Eschar		20.23 ± 1.96	16.85 ± 2.01
Unburned Skin	4.88 ± 0.16	4.98 ± 0.14	2.81 ± 0.30
Total Skin	4.88 ± 0.16	10.33 ± 0.53	8.85 ± 0.77
<hr/>			
	Mg Albumin/ml		
Plasma	26.04 ± 1.24	24.30 ± 0.99	7.98 ± 0.49

Values are means ± S.E.M. Mean rat weights (6 days postburn) were Control, 196 gm; Burned, 209 gm; Burned-Infected, 149 gm. Preburn weights were 180-200 gm.

1. Brown WL, Bowler EG, Mason AD, Jr, Pruitt BA, Jr: Protein metabolism in burned rats. Amer J Physiol 231:476-482, 1976.

Both the wet weight and dry weight of the skin from the burned area were greater than that of an equivalent area of skin from unburned rats. The cumulative result of these changes is shown in Table 2. The skin of the whole 20% body surface burned area contained 4.68 ml more water and 216 mg more albumin than did an equivalent area of skin of control rats.

Table 2. Comparison of Burned and Unburned Skin of 200 gm Rats

	Wet Weight Gm	Dry Weight Gm	Gm Water in Tissue	Mg Albumin in Tissue
Whole eschar (20% BS burn)	14.16	4.18	9.99	256.95
Control skin of equal area*	8.42	3.11	5.31	41.10
Difference (Burn-Control)	+5.74	+1.07	+4.68	+215.85

* Control rats were placed in the burning mold and the outline of the area was marked on the skin with ink. That area, which was equivalent to that of a 20% BS burn, was excised, weighed, and dried to constant weight. Values are means of 3 rats each group.

Additional groups of rats with a 20% body surface burn were untreated or were given a subcutaneous injection of hyaluronidase (150 units in 1 ml saline) or of 1 ml 0.15 M NaCl into the burn wound at one hour postburn. Rats of all 3 groups showed lowered plasma volumes at 6 hours postburn. When given free access to food and water, but no further treatment, the plasma volumes of all 3 groups returned to normal by 24 hours and were greater than normal at 48 hours postburn.

Water content of the burned skin of these rats was elevated at 6 hours postburn and was similar for all groups. The water content of the burned skin of all of the rats was still elevated at 24 hours and at 48 hours, but it was consistently lower in the burned skin of the rats which had been injected with hyaluronidase. This data will be reported later.

PRESENTATIONS

Brown WL: Albumin in burned rats. 8th Annual Meeting American Burn Association, San Antonio, Tx. 2 April 1976.

PUBLICATIONS

Brown WL, Bowler EG, Mason AD, Jr., Pruitt BA, Jr.: Protein metabolism in burned rats. Amer J Physiol 231:476-482, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION#	2. DATE OF SUMMARY*	REPORT CONTROLL. SYMBOL DD-DRAE(AR)436	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. RESEARCH*	8A. ORIGIN INSTR*	8B. SPECIFIC DATA - C. TRACTOR ACCESS	9. LEVEL OF DOW A. WORK UNIT
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61102A	3A161102E71R	01	300			
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code)* (U) Evaluation of Gastrointestinal Absorption and Nutritional Efficacy of Standard High Protein Diet in Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA*							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
69 07		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCE* ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				FISCAL YEAR		b. FUNDS (in thousands)	
a. DATES/EFFECTIVE:				76		.7	
b. NUMBER:				7T		23	
c. TYPE:				CURRENT		7	
d. KIND OF AWARD:							
e. CUM. AMT.							
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				Burn Study Branch			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: Douglas W. Wilmore, MD			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-5712			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: John F. Peterson, 1LT, MSC			
				NAME:			
23. KEYWORDS (Precede Each with Security Classification Code)							
(U) Gastrointestinal absorption; (U) High protein diet; (U) Trace elements; (U) Humans							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Precede rest of each with Security Classification Code.)							
23. (U) To evaluate the nutritional efficacy of standard high protein diets, nutritional supplements and elemental diets in burned patients. To determine the hormonal and dietary factors which influence nitrogen balance in thermally injured troops.							
24. (U) Use of defined diets or specific nutrient loading administered by the oral or intravenous route and determine nitrogen balance, amino acid patterns, or serum protein response.							
25. (U) 75 07 - 76 09 Nitrogen balance in burned man plateaus when the metabolic rate--carbohydrate ratio approaches unity. Addition of exogenous insulin can effect further nitrogen sparing. Phenylalanine flow increases in the postburn period as an index of muscle catabolism.							

*Available to contractors upon originator's approval

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 66
AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

TERMINATION REPORT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL
EFFICACY OF STANDARD HIGH PROTEIN DIETS IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

Douglas W. Wilmore, M.D.
John P. Peterson, Captain, AMSC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN DIETS IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Douglas W. Wilmore, M.D.
John P. Peterson, Captain, AMSC

Reports Control Symbol MEDDH-288(R1)

Improved and sustained dietary intake has continued as a major priority in supporting thermally injured patients. Additional dietary supplements and tube feedings have been developed in the metabolic kitchen by modifying commercially available liquid diet formulas. These enteral feedings are supplemented by hypercaloric glucose and amino acid infusions with or without fat emulsions. The specific role of dietary tyrosine and gastrointestinal absorption of other nutrients in injured patients have been studied and are presented in separate reports.

Gastrointestinal absorption
High protein diets
Trace elements
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^b	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^c	6. WORK SECURITY ^d	7. REGRADING ^e	8. DISSEM INSTR ^f	9. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
75 07 01	K. COMP	U	U	NA	NL	A. WORK UNIT	
10. NO./CODES ^g		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		61102A	3A161102B71R	01	119		
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Proceed with Security Classification Code) ^h (U) A Therapeutic Trial of Antacid in Prevention of the Clinical Complications Associated With Gastric Mucosal Disease in Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁱ 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 01		76 06		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER ^j				FISCAL YEAR		76	
c. TYPE:				CURRENCY		.6	
d. KIND OF AWARD:				f. CUM. AMT.		20	
20. RESPONSIBLE JOG ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^k US Army Institute of Surgical Research				NAME ^k US Army Institute of Surgical Research			
ADDRESS ^k Fort Sam Houston, Texas 78234				ADDRESS ^k Burn Study Branch Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish LEAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME ^l Albert J. Czaja, MD			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-5712			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: David K. Teegarden, MAJ, MC			
				NAME: Basil A. Pruitt, Jr, COL, MC			
				DA			
22. KEYWORDS (Proceed with Security Classification Code) (U) Curling's Ulcer; (U) Burned soldiers; (U) Gastritis; (U) Antacid							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Proceed text of each with Security Classification Code.)							
23. (U) To determine if neutralization of hydrogen ions by antacid administration to burned soldiers who manifest disruption of the gastric mucosal barrier will prevent progressive gastric mucosal damage, gastric hemorrhage, or perforation.							
24. (U) All patients admitted to the Institute of Surgical Research Within 72 hours after sustaining greater than 35% total body surface injury will be considered for this study. A lithium flux test will be performed as has been previously described within the 72-hour postburn period. The patients will then be randomly assigned to receive a standard antacid preparation or no neutralization of gastric acid. The patients with disruption of the gastric mucosal barrier who receive antacid therapy will be statistically compared as regards hemorrhage and perforation to those with disruption of the gastric mucosal barrier who receive no antacid therapy.							
25. (U) 75 07 - 76 09 This project is now completed. Conclusions from the data show that antacids significantly reduce gastroduodenal mucosal complications following thermal injury when compared to a nontreatment group. Of 27 patients treated with antacids, only one complication developed. However, of the 27 patients in the non-treatment group, seven major complications, including perforation of the gastrointestinal tract and significant hemorrhage occurred.							

^a Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 66
AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

FINAL REPORT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

**REPORT TITLE: A THERAPEUTIC TRIAL OF ANTACID IN PREVENTION
OF THE CLINICAL COMPLICATIONS ASSOCIATED WITH
GASTRIC MUCOSAL DISEASE IN BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**David K. Teegarden, M.D., Major, MC
Joseph C. McAlhany, Jr., M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: A THERAPEUTIC TRIAL OF ANTACID IN PREVENTION OF THE CLINICAL COMPLICATIONS ASSOCIATED WITH GASTRIC MUCOSAL DISEASE IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: David K. Teegarden, M.D., Major, MC
Joseph C. McAlhany, Jr., M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

This study prospectively investigated the effectiveness of antacids in the prevention of stress ulcerations following thermal injury.

The integrity of the gastric mucosal barrier (GMB) was assessed, using the lithium flux technique. Twenty one of the 54 study patients had abnormal GMB's. Twenty-seven patients received 60 cc of an antacid preparation containing a combination of aluminum hydroxide and magnesium hydroxide each hour; the remaining 27 received no antacid therapy. Clinical complications were compared in the two groups (Table 1).

TABLE 1

	Antacid Group	No Antacids
Total patients	27	27
Complications	7	1
Gastrointestinal hemorrhage	6	1
Perforation	1	0

Conclusions

As can be noted in Table 1, the antacid group demonstrated a markedly decreased incidence of gastrointestinal complications when compared to the nontreatment group. Based on this data, this unit currently administers antacids for prophylaxis in the prevention of erosive gastritis and ulceration following thermal injury.

PRESENTATIONS AND/OR PUBLICATIONS

McAlhany, J. C., Jr.: Antacid Control of Complications from Acute Gastroduodenal disease after burns. The American Association for the Surgery of Trauma, Scottsdale, Arizona, 11 September 1975.

PUBLICATIONS

McAlhany, J. C., Czaja, A. J., and Pruitt, B. A., Jr.: Antacid control of complications from acute gastroduodenal disease after burns. J Trauma 16:645-649, 1976.

Curling's ulcer
Burned soldiers
Antacid
Gastritis

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-
SURGERY

REPORT TITLE: PULMONARY COMPLICATIONS IN THERMAL INJURY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

Victor Lam, MD, Major, MC
James K. Sims, MD, Major, MC
Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
Peter A. Petroff, Jr, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A1611Q2B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-
SURGERY

REPORT TITLE: PULMONARY COMPLICATIONS IN THERMAL INJURY

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Victor Lam, MD, Major, MC
James K. Sims, MD, Major, MC
Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
Peter A. Petroff, Jr, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

A review of 39 completed patient records from July to September 1976 indicates that pulmonary complications continue to be a common problem in the burned patient. Of major interest to the clinician are the pathophysiology, early detection, and therapy of pulmonary injury associated with burns. Previous pulmonary studies in the burned patient utilized tests developed to quantify chronic obstructive lung and restrictive lung disease. These conventional measurements are inadequate for early detection of pulmonary edema, inhalation injury, and upper airway obstruction. In the next year, more specific methods will be required to evaluate alternate therapies in the pulmonary complications of the burned patient.

Inhalation injury
Thermal injury
Pulmonary complications
Therapy

PULMONARY COMPLICATIONS IN THERMAL INJURY

A review of 39 completed patient records from July to September 1976 indicates that pulmonary complications continue to be a common problem in the burned patient. Of major interest to the clinician are the pathophysiology, early detection, and therapy of pulmonary injury associated with burns.

Incidence of Inhalation Injury

Previous reports of the incidence of inhalation injury by standard clinical criteria is about 3%. With the advent of fiberoptic bronchoscopy and $^{133}\text{Xenon}$ lung scans, the diagnosis of inhalation injury due to toxic fumes and/or products of incomplete combustion can be made earlier in the hospital course. Of the 39 patients reviewed, 15 had the diagnosis of inhalation injury for a 38% incidence. Fiberoptic bronchoscopy was performed at bedside on 20 patients and xenon scans, as a diagnostic procedure, on 12.

For inhalation injury, the clinical problems are accurate early diagnosis, possible acute upper airway obstruction, and therapeutic options. For inhalation injury with smoke inhalation, upper respiratory tract findings may not indicate degree of pulmonary parenchymal involvement. Serial determination of pulmonary interstitial fluid volume may be of prognostic usefulness.

Our current therapeutic regimen for inhalation injury includes intermittent positive pressure breathing, mucomyst, chest physiotherapy, occasional steroids, bronchodilators, and humidified oxygen. Aerosolized Gentamycin has been used according to protocol study. Among other possible therapeutic options available for evaluation are prophylactic CPAP, incentive spirometry, and synthetic surfactant. The current therapy is nonspecific and has not been evaluated in a prospective double-blind clinical study. At this time, there is little scientific evidence for the efficacy of IPPB in pulmonary disease.

Acute Upper Airway Obstruction

Acute upper airway obstruction has necessitated emergency airway management in six cases in the past 15 months and in two cases in the 39 cases reviewed.

The use of steroids has been advocated. However, quantitative evaluation of upper airway obstruction has been difficult.

Proposed methods for evaluation of upper airway obstruction include the use of ultrasound probe to measure trachea dimensions and oscillometric resistance measurements to determine nasal and tracheal resistance with gases of different viscosity.

Acute Respiratory Failure

Of 39 patients reviewed, 17 required the ventilatory support of a volume-cycled respirator during their hospitalization. All except three patients with tracheostomies were managed via an endotracheal tube. Many patients required high airway pressures and post-expiratory pressure to maintain adequate ventilation and arterial oxygenation. The low pulmonary compliance was due in part to increased interstitial pulmonary fluid or pulmonary edema.

Postmortem findings on eight patients showed pulmonary edema and congestion, indicating fluid overload (with respect to pulmonary parenchyma) is a major factor in the pathogenesis of acute respiratory distress syndrome in these patients. It is suspected but not proven that increased capillary permeability plays a major role; however, other possibilities include the role of vasoactive substances and inhomogenous distribution of arteriolar constriction because of hypoxemia. Early measurement of pulmonary interstitial fluid volume would be useful in fluid management in patients prone to the acute respiratory distress syndrome.

Sulfamylon Topical Therapy

Three patients were suspected of having an adverse reaction to Sulfamylon topical therapy with metabolic alkalosis and hyperventilation. Despite the intermittent application and rotational use of Sulfamylon, the pulmonary complications continued to occur, perhaps in predisposed patients. Earlier detection of Sulfamylon hypersensitivity would be clinically valuable. Carbon dioxide respiratory center testing may allow differentiation of susceptible patients.

Other Complications

There were no reports of pleural effusion or pulmonary emboli in the period reviewed. Perhaps the absence of pulmonary emboli in this seemingly susceptible class of almost immobile patients is a protective result of a hyperkinetic metabolic effect on circulation. Pneumothorax occurred in three patients with two cases of iatrogenic nature, secondary to central intravenous line insertion. Twelve patients had the clinical and laboratory diagnosis of bacterial pneumonia, including two cases of hospital-acquired aspiration pneumonia.

Inhalation Injury and Surfactant Therapy

Inhalation injury, associated with pulmonary failure, adds significantly to the morbidity and mortality of thermal injury. Even in the absence of inhalation injury, previous research has shown a significant decrease in pulmonary surfactant.

In this study, tracheostomized goats were subjected to smoke inhalation. Dynamic compliance prior to injury was compared to dynamic compliance following treatment with nebulized dipalmityllecithin or with the DPL solvent.

Histopathology showed inhalation injury in all animals; no post-treatment difference between the two groups was observed. The dynamic compliance, however, in the surfactant group increased 67.8% over control values while it decreased 9.58% in the solvent-treated group. Although the number of animals was too small to achieve statistical significance, this experiment raises interesting possibilities as to the use of surfactant in the treatment of inhalation injury.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMARY ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9. SPECIFIC DATA- CONTRACTOR ACCESS ^a	10. LEVEL OF SUM ^a
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61102A		3A161102B71R		01	
b. CONTRIBUTING						316	
c. CONTRIBUTING							
11. TITLE (Proceed with Security Classification Code) ^a (U) Evaluation of Dopamine (3,4-Dihydroxyphenylethylamine) For Treatment of Septic Shock in Burned Troops (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 02		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
Not Applicable				a. PROFESSIONAL MAN YRS			
a. DATE/EFFECTIVE:		EXPIRATION:		b. PRESENT		c. FUNDS (in thousands)	
b. NUMBER: ^a				76		.6	
c. TYPE:		d. AMOUNT:		77		.2	
e. KIND OF AWARD:		f. CUM. AMT.		YEAR		6	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a US Army Institute of Surgical Research				NAME: ^a US Army Institute of Surgical Research			
ADDRESS: ^a Fort Sam Houston, Texas 78234				ADDRESS: ^a Clinical Division Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: ^a Gary Welch, LTC, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-5712			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Douglas W. Wilmore, MD			
				NAME:			
				DA			
22. KEYWORDS (Proceed with Security Classification Code)							
(U) Dopamine; (U) Septic shock; (U) Burn injury; (U) Cardiac output; (U) Humans							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Proceed text of each with Security Classification Code.)							
23. (U) To evaluate the use of dopamine in treatment of septic shock in burned soldiers.							
24. (U) Patients meeting the criteria of reduced urine output, hypotension, and evidence of reduced central perfusion will have a Swan-Ganz thermal dilution cardiac output catheter inserted for determination of cardiac output. After baseline studies have been obtained, an infusion of dopamine will be administered intravenously at a rate to raise mean arterial blood pressure to 60 torr. Cardiac outputs will then be determined again.							
25. (U) 75 07 - 76 09 Eleven patients received intravenous dopamine. Of the 11, only one survived the septic episode. Dopamine produced an increased cardiac index and reduced peripheral resistance at low doses. Higher doses also increased cardiac index and raised the systemic resistance when compared to low dose administration.							

FINAL REPORT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EVALUATION OF DOPAMINE (3,4-DEHYDROXYPHENYL-ETHYLAMINE) FOR TREATMENT OF SEPTIC SHOCK IN BURNED TROOPS

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
Charles Drueck III, MD
Robert W.J. Baird, MD, Major, MC
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Reports Control Symbol: MEDDH-283(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EVALUATION OF DOPAMINE (3,4-DEHYDROXYPHENYL-ETHYLAMINE) FOR TREATMENT OF SEPTIC SHOCK IN BURNED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
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Robert W.J. Baird, MD, Major, MC
Douglas W. Wilmore, MD

Eleven extensively burned patients with an average age of 30.2 years and an average burn of 62.5% of the total body surface who developed septic shock have been treated with dopamine in dosage ranging from 4.6 - 46.1 mcg/kg/min. This agent further elevated an already increased cardiac index in these patients with little effect on mean arterial blood pressure. Pulmonary vascular resistance was slightly elevated while systemic vascular resistance was decreased with low doses of dopamine and essentially unchanged with higher doses. Both left ventricular and right ventricular stroke work were significantly increased following administration of dopamine. Only one of the 11 patients survived despite the improvement in cardiac performance brought about by dopamine. The high mortality in this group of patients reflects an inability to eliminate the source of sepsis in the patients studied.

Dopamine
Septic shock
Burn injury
Cardiac output
Humans

EVALUATION OF DOPAMINE (3,4-DEHYDROXYPHENYLETHYLAMINE) FOR TREATMENT OF SEPTIC SHOCK IN BURNED TROOPS

Sepsis remains the most frequent cause of death in extensively burned patients. Septic shock, which occurs frequently in patients with gram-negative infections, is often refractory to treatment by fluid loading, which may only precipitate pulmonary edema or the administration of sympathomimetic agents, such as catecholamines, which may cause oliguria and even acute tubular necrosis. The use of a new vasoactive agent, dopamine, has been evaluated in a group of burn patients by assessing its ability to improve cardiac function while maintaining adequate peripheral and renal blood flow.

Methods

Eleven patients with burns of more than 40% of the total body surface who developed hypotension and oliguria, unresponsive to volume loading, in the presence of a positive blood culture, have been studied (Table 1). Prior to the administration of any pressor agents, a Swan-Ganz 7f flow-directed thermal dilution cardiac output catheter was inserted either percutaneously or via a cutdown incision. If the patient's condition permitted a preinfusion, 2-hour creatinine clearance was done. All pressures were transduced with a Trantec pressure transducer and displayed on a Tektronix 412 physiologic monitor. Cardiac outputs were done using an Instrumentation Laboratory thermal dilution cardiac output computer. Arterial pressures were determined by either a direct intra-arterial catheter or the infrasonde blood pressure monitor. Simultaneous systolic time intervals were recorded on an 8-channel Electronics for Medicine recorder, using standard limb leads for the EKG, a Statham strain gauge for the carotid pulse tracing, and a Hewlett Packard microphone for the phonocardiogram. Following baseline studies, dopamine infusion was begun at a dose of 4.6 mcg/kg/min. The studies noted above were then repeated. The dopamine infusion was then increased in 400 mcg/min increments to a maximum of 46.1 mcg/kg/min until the desired response was obtained, another agent was used, or the patient expired.

Results

Two hundred thirty-six serial determinations of cardiac output, pulmonary artery pressure, wedge pressure, central venous pressure, blood pressure and pulse rate were performed before (Table 2) and during dopamine administration. The dopamine dosage employed ranged from 4.6 to 46.1 mcg/kg with an average low dose of 7.25 mcg/kg/min and an average high dose of 24.2 mcg/kg/min. When given in low doses, dopamine increased the cardiac index, pulmonary vascular resistance, and left ventricular stroke work. These changes were accompanied by a decrease in systemic vascular

TABLE 1

DOPAMINE IN SEPTIC SHOCK

PATIENTS			
	TOTAL	LOW DOSE	HIGH DOSE
NUMBER	11	8	9
SURVIVORS	1	0	1
MALE	7	5	5
FEMALE	4	3	4
AVERAGE AGE	30.18 ± 3.70	30.38 ± 5.18	28.33 ± 3.02
AVERAGE % BURN	62.45 ± 3.49	58.81 ± 3.65	64.06 ± 3.73
AVERAGE % 3°	34.64 ± 5.74	31.12 ± 7.27	32.22 ± 6.80

TABLE 2
DOPAMINE IN SEPTIC SHOCK

	NORMAL	PRE-PRESSOR	% CHANGE
C.I. (L/min/M ²)	3.2 + .2	4.95 + .77	+ 55%
MABP (mm Hg)	80 +5	51.75 +5.34	- 42%
SVR $\frac{\text{Dynes-sec}}{\text{cm}^5 \cdot \text{M}^2}$	2180 +210	707.67 +93.71	- 68%
PVR $\frac{\text{Dynes-sec}}{\text{cm}^5 \cdot \text{M}^2}$	270 +45	156 +30.68	- 42%
LVSW $\frac{\text{Gram Meter}}{\text{M}^2}$	56 +6	30.30 +5.39	- 46
RVSW $\frac{\text{Gram Meter}}{\text{M}^2}$	8.76 +2	8.98 +1.9	+ 2%

resistance to an average of 17% below prepressor levels, with the lowest value recorded being 66 dynes sec/cm⁵ · M². The right ventricular stroke work increased significantly, reflecting the increase in pulmonary vascular resistance. In high doses, the cardiac index, systemic vascular resistance and the pulmonary vascular resistance increased, although none of the increases were statistically significant. The left and right ventricular stroke work increased significantly, reflecting the elevated cardiac index and increased vascular resistances. The mean arterial blood pressure increased by 23%.

Comparison of the response to low dose dopamine with high dose dopamine revealed that the higher dose increased systemic vascular resistance and left ventricular stroke work (Table 3). The 27% increase above low dose levels observed with the higher doses represented only a 5% increase of vascular resistance above prepressor levels. A 21% increase of mean arterial blood pressure was associated with administration of the higher doses of dopamine.

Low dose dopamine caused a 17% decrease in systemic vascular resistance in association with a 35% increase in pulmonary vascular resistance, with no effect on mean arterial blood pressure. In high doses, dopamine increased both systemic vascular resistance and cardiac index, resulting in an increase in average mean arterial blood pressure to 64 mm Hg, a 23% increase over prepressor levels.

Discussion

In these 11 study patients with extensive burns and documented sepsis, hypotension, indicated by a mean arterial blood pressure 42% less than predicted, appears directly related to the altered vascular resistance, which was 68% below predicted. An incomplete compensation has been achieved in such patients by elevation of the cardiac index to levels averaging 55% above predicted values. This compensation, if untreated, will lead to progressive cardiovascular failure. When volume loading failed to ameliorate these alterations, dopamine was started to preserve renal blood flow while increasing vascular resistance and supporting cardiac function to reverse the shock state. In both high and low dosage, dopamine increased cardiac index, mean arterial blood pressure and pulmonary vascular resistance, although the elevations did not attain statistical significance. Low dosage dopamine appeared to decrease systemic vascular resistance.

The effect of dopamine infusion on the pulmonary resistance may be an important consideration in patients suffering from respiratory insufficiency and hypotension. An increase in resistance by redistributing pulmonary blood flow may actually increase ventilation-perfusion abnormalities. Dopamine produced a rise in pulmonary vascular resistance, even though pulmonary resistance was below normal prior to dopamine infusion, and this effect may limit

TABLE 3
DOPAMINE IN SEPTIC SHOCK

	PRE PRESSOR	LOW DOSE DOPAMINE (7.25 \pm 0.52 mcg/kg/min)	HIGH DOSE DOPAMINE (24.2 \pm 3.1 mcg/kg/min)
C.I. (L/min/M ²)	4.95 \pm .77	5.44* \pm .43	5.48 \pm .39*
MABP (mm Hg)	51.75 \pm 5.34	52.88 \pm 5.74*	64.08 \pm 5.912
SVR <u>Dynes-sec</u> <u>cm⁵.M²</u>	707.62 \pm 93.71	584.88 \pm 68.01*	745.38 \pm 65.31*
PVR <u>Dynes-sec</u> <u>cm⁵.M²</u>	156 \pm 30.68	210.62 \pm 57.07*	189 \pm 23.45*
LVSF <u>Gram Meter</u> <u>M²</u>	30.30 \pm 5.39	36.68 \pm 4.65*	40.80 \pm 6.012
RVSF <u>Gram Meter</u> <u>M²</u>	8.98 \pm 1.90	14.25 \pm 2.822	13.63 \pm 1.822

* No significance

1 . p .01

2 . p .05

the clinical usefulness of the agent. In both low doses and high doses, dopamine significantly increased both left ventricular and right ventricular stroke work with this inotropic effect predominating and bringing about a transient improvement in the hemodynamic status of these patients.

This study has demonstrated that dopamine is capable of improving cardiac function in septic burn patients. Only at the higher dose levels, however, did it increase systemic vascular resistance and even then the elevation was not to significant levels. The decreased systemic vascular resistance characteristic of septic shock was, in fact, exaggerated by infusion of low doses of dopamine, which would, therefore, appear to offer no unique benefit to the septic burn patient. The mortality of 91% in this group of patients (one of 11 survived) reflects not the ineffectiveness of dopamine but an inability to eliminate the source of sepsis and the persistence of the deleterious effects of the septic process.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OF 6395	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUM. ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. ORIGIN INSTN ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS ^a	10. LEVEL OF SUM ^a
75 07 01	H. TERM	U	U	NA	NL	<input type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
A. PRIMARY	61102A	3A161102B71R		01	305		
B. CONTRIBUTING							
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Prolongation of Skin Allograft Survival by Immunosuppressive Therapy (Upjohn ATG) in Soldiers With Massive Thermal Injury (44)							
12. SCIENTIFIC / NO TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 04		76 09		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE. EXPIRATION:				PRECEDING		B. FUNDS (in thousands)	
B. NUMBER ^a				76		.5	
C. TYPE				FISCAL		7	
D. AMOUNT				YEAR		CURRENT	
E. KIND OF AWARD				F. CUM. AMT.			
18. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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FOREIGN INTELLIGENCE NOT CONSIDERED				SOCIAL SECURITY ACCOUNT NUMBER			
				ASSOCIATE INVESTIGATORS			
				NAME: Gary Welch, LTC, MC			
				NAME: Douglas W. Wilmore, MD DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Autograft; (U) Mesh graft; (U) Excision of fascia; (U) Antithymocyte globulin; (U) Isolation; (U) Laminar flow room; (U) Homograft; (U) Humans							
23. TECHNICAL OBJECTIVE ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
<p>23. (U) The objective of this protocol is to see if burned soldiers with extensive injuries can be effectively treated with antithymocyte globulin causing suppression of cellular immunity and allowing prolongation of allograft take in areas of excision.</p> <p>24. (U) Patients in the 15 to 40 age group with full-thickness thermal injury greater than 60% of their body surface and without significant pulmonary injury will be immunosuppressed with antithymocyte globulin and serially excised and grafted with available autograft and then with fresh homograft. The homograft will be allowed to remain in place. The patient's immunosuppression will continue and as donor sites become available the allograft will be surgically excised and replaced with autograft. Immunosuppression will continue until all but 15% of the grafted surface is covered with autograft.</p> <p>25. (U) 75 07 - 76 09 To date two patients have been studied; a 25 year-old female with an 85% thermal injury and an 18 year-old female with a 90% thermal injury. Both underwent excision and immunosuppression; however, both had a rather rapid septic course. It has been elected to defer any more trials with the immunosuppression until a patient isolator in the form of a vertical laminar flow unit has been obtained for the unit.</p>							

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: PROLONGATION OF SKIN ALLOGRAFT SURVIVAL BY IMMUNOSUPPRESSIVE THERAPY (UPJOHN ATG) IN SOLDIERS WITH MASSIVE THERMAL INJURY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

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Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: PROLONGATION OF SKIN ALLOGRAFT SURVIVAL BY IMMUNOSUPPRESSIVE THERAPY (UPJOHN ATG) IN SOLDIERS WITH MASSIVE THERMAL INJURY

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78134

Period covered in this report: 1 July 1975 - 30 September 1976

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Reports Control Symbol MEDDH-288(R1)

The object of this study is to evaluate the prolongation of survival of allograft which has not been tissue typed. Massive burn wounds fatal by historic comparison, are to be excised in stages and covered with non-typed allograft from cadaver source. The patients are then immunosuppressed with antithymocyte globulin and the grafts allowed to take and remain in place. As donor sites become available the allograft is excised and replaced with isograft. This has been a feasible treatment in children with extensive burns. To date at the Institute of Surgical Research two patients have been managed in this fashion, neither has been a survivor and both have died of sepsis.

At present we are awaiting installation of a laminar flow controlled environment unit in order to better isolate the patient prior to further attempts. However it must be noted that we see very few patients with greater than 65 to 70% third degree burn without significant inhalation injury. The study will be resumed when the laminar flow capability has been acquired.

Autograft
Mesh graft
Excision of fascia
Antithymocyte globulin

Isolation
Laminar flow room
Homograft
Humans

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a		2 DATE OF SUMMARY ^a		REPORT CONTROL SYMBOL	
				DA OG 6955		76 10 01		DD DR&E(AR)636	
3 DATE PREV SUMMARY	4 KIND OF SUMMARY	5 SUMMARY SCTY ^a	6 WORK SECURITY ^a	7 REGRADING ^a	8A DISSEM INSTR ^a	8B SPECIFIC DATA CONTRACTOR ACCESS		9 LEVEL OF SUM	
76 10 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		A. WORK UNIT	
10 NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY		61102A		3A161102B71R		01		122	
b. CONTRIBUTING									
c. CONTRIBUTING									
11 TITLE (Precede with Security Classification Code) ^a									
(U) The Effect of Dextrose and Amino Acid Solutions on Nitrogen Excretion and Nitrogen Balance Following Thermal Injury in Burned Soldiers (44)									
12 SCIENTIFIC AND TECHNOLOGICAL AREAS ^a									
003500 Clinical Medicine									
13 START DATE			14. ESTIMATED COMPLETION DATE			15 FUNDING AGENCY		16 PERFORMANCE METHOD	
75 11			76 09			DA		C. In-House	
17 CONTRACT/GRANT									
Not Applicable									
a. DATES/EFFECTIVE:				b. EXPIRATION:				c. FUNDING (In thousands)	
								17	
d. NUMBER ^a				e. AMOUNT ^a				10	
f. TYPE:				g. CUM. AMT.					
h. KIND OF AWARD									
19 RESPONSIBLE JOG ORGANIZATION				20 PERFORMING ORGANIZATION					
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research					
ADDRESS ^a Fort Sam Houston, Texas 78234				ADDRESS ^a Fort Sam Houston, Texas 78234					
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)					
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21 GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER					
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS					
				NAME ^a Clement L. Slade, CPT, MC					
				NAME ^a Douglas W. Wilmore, MD DA					
22 KEYWORDS (Precede EACH with Security Classification Code) (U) Nitrogen balance; (U) Thermal injury; (U) Dextrose; (U) Amino acid solutions; (U) Humans									
23 TECHNICAL OBJECTIVE, 24 APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)									
<p>23. (U) To determine the efficacy of dextrose administration as opposed to protein administration in the form of amino acid solutions. The effect that one or both of these solutions have on nitrogen balance will be determined and evaluation will be made of the solution or combination of solutions which will be most appropriate for preservation of nitrogen balance in the thermally injured soldier.</p> <p>24. (U) The patients sustaining greater than 30% total body surface area burn who are admitted to the Institute of Surgical Research will be deemed suitable for the study providing they have no prior history of liver disease, intestinal disorders or other chronic debilitating diseases. These patients will be randomized and they will receive either dextrose alone and will have daily 24-hour urine clearances for determination of nitrogen loss or they will receive dextrose plus amino acid solutions or amino acid solution alone. The routine bloods which are normally drawn will provide the necessary serum information. The routine urine collections which are obtained will be analyzed for total nitrogen and amino acid excretion levels in addition to those substances which are normally obtained.</p> <p>25. (U) 75 11 - 76 09 Twenty-two patients with body surface thermal injuries of 30% or greater have thus far been studied. The initial data indicates that calorie per calorie low dose glucose and low dose amino acid solutions achieve similar effects as nitrogen sparing. No support has been gained for the hypothesis that amino nitrogen alone provides a better nitrogen balance. Similar findings were apparent in septic patients with respect to nitrogen sparing; however, cardiac output and liver function as measured by the Indocyanine green excretion test were markedly altered in a pathologic way in patients receiving only amino acid solutions. These defects could be corrected by adding glucose to the infusate. Currently, studies are being conducted to determine the proper mixture.</p>									

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECT OF DEXTROSE AND AMINO ACID SOLUTIONS ON
NITROGEN EXCRETION AND NITROGEN BALANCE FOLLOWING
THERMAL INJURY IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 July 1975 - 30 September 1976

Investigators:

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With the technical assistance: Calvin P. Kenriedy

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECT OF DEXTROSE AND AMINO ACID SOLUTIONS
ON NITROGEN EXCRETION AND NITROGEN BALANCE FOLLOWING
THERMAL INJURY IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: W. Scott McDougal, M.D., Major, MC
Douglas W. Wilmore, M.D. .

Report Control Symbol MEDDH-288 (R1)

Near isotonic hypocaloric diets containing glucose and amino acids significantly diminish nitrogen loss in severely burned patients. The impact of both substrates on nitrogen balance was similar and additive. Indices of organ function improved with glucose addition to the diet. Glucose, together with amino acids, is the most efficacious hypocaloric diet for short-term alimentation in the critically ill patient.

Nitrogen balance
Thermal injury
Dextrose
Amino acid solutions
Humans

THE EFFECT OF DEXTROSE AND AMINO ACID SOLUTIONS ON NITROGEN EXCRETION AND NITROGEN BALANCE FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

Thermal injury, like trauma of other etiologies, results in a catabolic state which must be limited and ultimately reversed if recovery is to occur. A positive nitrogen balance may be obtained by the provision of adequate protein and calories. Unfortunately, in the immediate post traumatic period, gut motility is impaired and if nutrients are to be given, they must be administered intravenously.

Isosmotic solutions which provide calories are useful in limiting nitrogen loss. Such solutions may be administered by peripheral vein and are particularly advantageous in situations where lack of oral intake is of limited duration, difficult fluid and electrolyte problems require frequent alterations in infusion rate or electrolyte composition or frequent administration of intravenous medications is required. The purpose of this study is to define the composition of a near isosmotic solution with respect to amino acid and/or glucose content which will provide maximal effect per calorie in reducing nitrogen loss and preserving optimal organ and metabolic functions in traumatized patients with and without systemic infection.

MATERIALS AND METHODS

Thirty-two thermally injured patients with an average burn size of $54.7 \pm 18.3\%$ and a mean age of 35.2 ± 7.2 years were resuscitated with 3 to 4 cc lactated Ringer's per per cent burn per body weight in kilograms. Immediately following the 24 hour resuscitative period, the patients were divided into three groups. Nutrients were given isosmotically by vein and caloric intake was not permitted by mouth during the three to nine day study period. The first group (Group I) received glucose free infusates either devoid of calories or containing amino acids (FreAmine II, McGaw). Each patient was maintained on a constant dose for a 24 hour period; however, the quantity of amino acids administered on a day to day basis was randomly varied.

The second group (Group II) was given either 60 or 120 grams glucose per m^2 per 24 hours or 60 or 120 grams of glucose per m^2 per 24 hours plus amino acids (Fre Amine II, McGaw). The quantity of amino-nitrogen administered was maintained at a constant infusion rate for 24 hour periods; however, the amount of amino acids given from day to day was varied in a periodic random manner.

The third group (Group III) received 60 grams glucose plus approximately 11 grams amino-nitrogen (Fre Amine II, McGaw) per m^2 per 24 hours continuously for three to nine days.

When a positive blood culture was obtained, the patient was permanently excluded from the above groups. During the course of therapy,

4 patients from Group I, 10 patients from Group II, and 1 patient from Group III had positive blood cultures at the beginning and termination of a 24 hour infusion period. These bacteremic patients provided the data for analyzing the effect of various infusions in the presence of systemic infection.

Daily blood cultures were obtained in all patients throughout the study. Plasma was analyzed every 24 hours for chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (by the glucose oxidase method), calcium and inorganic phosphate utilizing the Auto-analyzer (Technicon); for sodium and potassium by flame photometry (International Laboratories 343); and for osmolality by freezing point depression (Advance Osmometer). Arterial blood was analyzed at least once a day for pH, pCO_2 , pO_2 and O_2 saturation (Corning 165).

Blood samples drawn at the midpoint and end of each 24 hour period were immediately separated and the plasma stored at -15 to -20 degrees centigrade until assayed. The samples were analyzed for insulin by a modification of the technique of Hales and Randle¹ and for free fatty acids by the method of Trout, Estes and Friedberg.² Plasma preserved with Trasylol and sodium EDTA drawn at the same times and stored in the same manner was analyzed for glucagon by the immunoassay method of Unger utilizing the 30 K antibody.³

Twenty-four hour urine collections were obtained throughout the study period and analyzed for sodium and potassium by flame photometry (International Laboratories 343), for chloride, creatinine, glucose (glucose oxidase method), calcium, inorganic phosphate, and urea nitrogen with the Auto-analyzer (Technicon) and for total nitrogen utilizing the sulfuric perchloric acid digested method of Kjeldahl.

Intake and output as well as body weight were recorded on a daily basis. Patients who were hypotensive or required a cardiotoxic drug to support blood pressure, had a prior history of diabetes, or had significant renal insufficiency were excluded from the study.

Calculations: Nitrogen balance was corrected for alterations in the blood urea nitrogen for each 24 hour period and expressed per square

1. Hales CN and Randle PJ: Immunoassay of insulin with insulin-antibody precipitate. *Biochem J* 88:137, 1963.

2. Trout DL, Estes EH, Jr., Friedberg SJ: Titration of free fatty acids of plasma: a study of current methods and a new modification. *J Lip Res* 1:199, 1966.

3. Faloona GR and Unger RH: Glucagon in "Methods of Hormone Radioimmunoassay", ed. Juffee BM and Behrman HR. Academic Press, New York and London, 1974, pp 317-330.

meter of body surface area. The difference between the BUN at the inception and the termination of the study period was multiplied by 0.60 by the body weight in kilograms. This provided an estimate of nitrogen retained by the patient not reflecting protein synthesis and assumes that urea distributes throughout the body water in a concentration equal to that of plasma. The quantity was either added or subtracted according to whether accumulation or loss occurred from the total nitrogen excreted in the urine. This quantity was subtracted from the nitrogen intake and the result divided by the body surface area in square meters.

The non-urea nitrogen excreted in the urine is composed of uric acid, ammonia, creatinine, amino acids and peptides. If urinary creatinine, ammonia and uric acid are relatively constant between groups as they were in this study, an estimate of the amino acids lost in the urine expressed as a function of those infused may be obtained by subtracting the urine urea nitrogen from the total nitrogen and dividing

the quantity by the amount of infused nitrogen ($\frac{TUN-UUN}{NIN}$). An expression of the nitrogen composition of the urine is provided by dividing the urine urea nitrogen by the total nitrogen excreted in the urine (UUN/TUN). Glomerular filtration rate, fractional sodium and fractional potassium excretions, sodium, potassium and water balance were calculated in the standard manner. Caloric load was determined by multiplying the grams of glucose and/or amino acids in the infusates by 4 Kcalories.

Linear regression lines expressing nitrogen balance as a function of nitrogen intake were determined by the method of least squares. Significant differences for the slopes of the lines as well as the population of points described by the regressions between the different groups was determined by the analysis of covariance. The student t-test was used to determine significant differences between groups of data.

Two matrices were formed, one for non-bacteremic and the other for bacteremic patients. Carbohydrate (glucose) doses of 0, 60 and 120 grams per m^2 formed one side of the matrix while amino-nitrogen doses of 0, 10 and 15 grams per m^2 formed the other. The nitrogen balance as a function of a combination of these doses was determined by calculation utilizing the appropriate regression equation which had been obtained from the data of Groups I and II.

RESULTS

Ten patients with an average total body surface area burn of $48.7 \pm 19.7\%$ and a mean age of 36.4 ± 19.5 years were infused with amino acid or electrolyte solutions devoid of glucose. Daily blood cultures were negative during each of the 24 hour balance periods depicted by the triangle's in Figure 1. The linear regression line illustrated in Figure 1 was fitted to the values by the method of least squares ($r^2 =$

.7407) and is described by the equation: $y = .7234x - 17.6798$.

Ten patients with a mean injury of $53.5 \pm 19.1\%$ of the body surface area and an average age of 33.6 ± 16.6 years were given 60 grams glucose per m^2 as the sole caloric load per 24 hours or 60 grams glucose plus 5.59 to 20.15 grams amino-nitrogen per m^2 per 24 hour period. Blood cultures were negative for each of the daily balance periods depicted by the X's in Figure 1. The linear regression line determined by the method of least squares illustrated in Figure 1 has an $r^2 = .8450$ and is described by the equation: $y = .6131x - 10.7070$.

Six patients who had sustained a $61.7 \pm 15.5\%$ total body surface area burn and had an average age of 47.3 ± 14.7 years were infused with 120 grams glucose per m^2 per 24 hours or 120 grams glucose plus 7.02 to 20.00 grams amino-nitrogen per m^2 per 24 hours. All blood cultures during each 24 hour balance period depicted by the circles in Figure 1 were negative. The linear regression line illustrated for the data in Figure 1 ($r^2 = .8769$) has the form of: $y = .5820x - 7.5343$.

The slopes of the linear regression lines for the three groups are not significantly different; however, each line represents a distinct population and is significantly different from each of the other two regressions by the analysis of covariance. (60 grams glucose + AA versus 120 grams glucose + AA, $p < .001$; AA versus 60 grams glucose + AA, $p < .001$; and AA versus 120 grams glucose + AA, $p < .001$).

Six patients with an average total body surface area burn of $59.6 \pm 16.6\%$ and a mean age of 24.7 ± 9.0 years were infused by peripheral vein with 60 grams glucose plus 11.32 ± 1.49 grams amino-nitrogen per m^2 per 24 hours continuously for three to nine days. All blood cultures up to and including the study period were negative. Twenty four hour nitrogen balances calculated on the final day of the study revealed an average balance of -3.80 ± 1.05 grams per m^2 . The value calculated from the linear regression equation of the 60 gram glucose plus amino-acid group for a nitrogen intake of 11.32 grams is -3.77 grams per m^2 . The similar values support the validity of 24 hour dose schedules in Groups I and II.

Four patients with a mean burn size of $43.5 \pm 7.6\%$ and an average age of 24.0 ± 8.1 years had positive blood cultures (one for *Serratia marcescens*, 3 for *Klebsiella pneumoniae*) at the beginning and termination of the 24 hour infusion period. These patients received glucose free solutions, containing either no amino-nitrogen or 5.69 to 18.99 grams amino-nitrogen per m^2 per 24 hours. Each 24 hour balance period is depicted by triangles in Figure 2. The linear regression line for the data ($r^2 = .6239$) illustrated in Figure 2 is described by $y = .4550x - 24.0010$.

Six patients who had sustained an average total body surface area burn of $50.3 \pm 16.5\%$ and had a mean age of 30.7 ± 16.3 years developed

Figure 1. Nitrogen balance per square meter body surface corrected for alterations in blood urea nitrogen as a function of nitrogen intake in nonbacteremic patients infused with either amino acids, 60 grams glucose per m^2 plus amino acids or 120 grams glucose per m^2 plus amino acids.

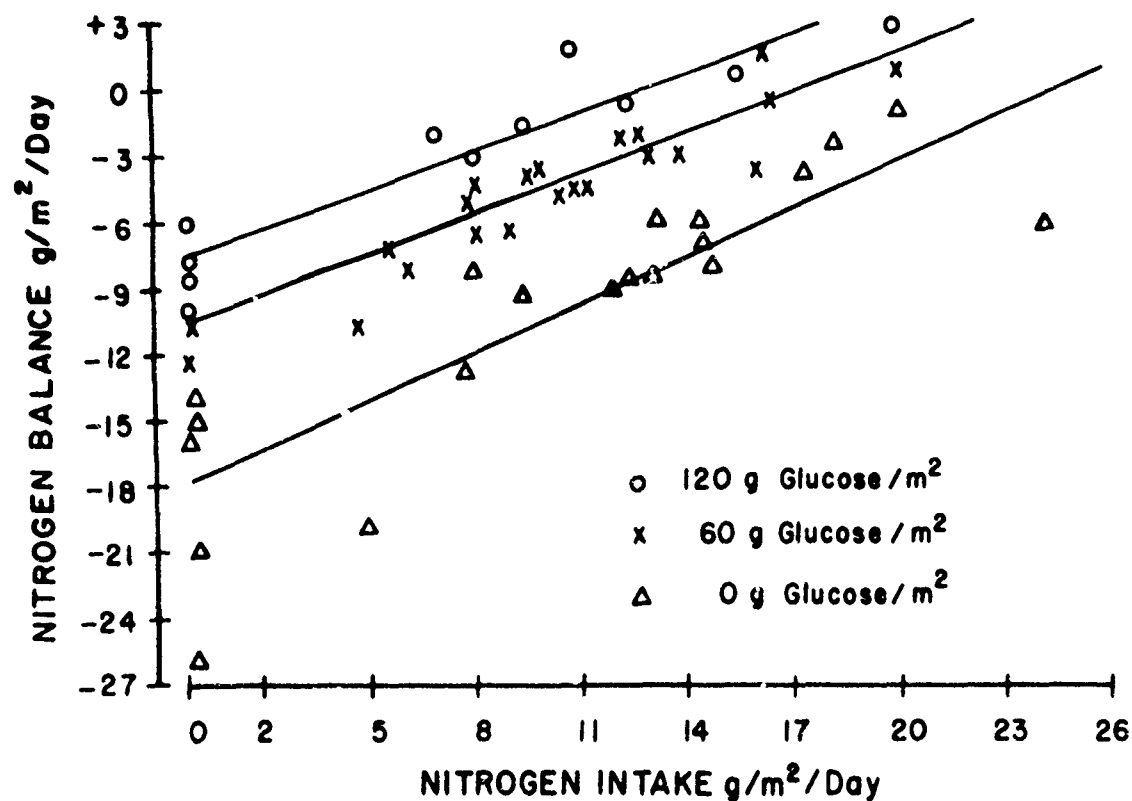
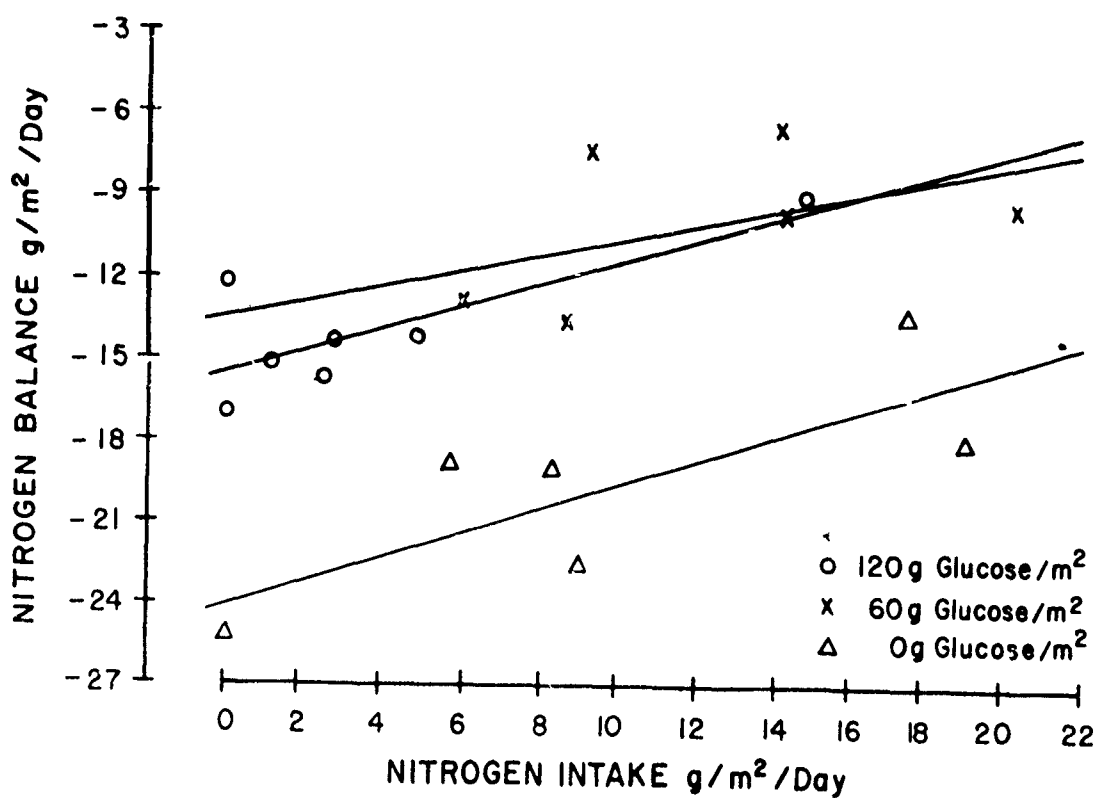


Figure 2. Nitrogen balance per square meter body surface corrected for alterations in blood urea nitrogen as a function of nitrogen intake in bacteremic patients infused with either amino acids, 60 grams glucose/ m^2 plus amino acids or 120 grams glucose/ m^2 plus amino acids.



positive blood cultures for *Klebsiella pneumoniae* at the inception and termination of a 24 hour study period in which they had received 60 grams glucose plus 6.03 to 20.15 grams amino-nitrogen per m^2 per 24 hours. The individual balance data for each 24 hour period is depicted by the X's in Figure 1. The linear regression line ($r^2 = .2524$) for the data is described by $y = .2817X - 13.3368$.

Five patients with an average burn of $66.6 \pm 15.4\%$ and an average age of 40.4 ± 16.8 years had positive blood cultures (one for *Staphylococcus aureus*, 4 for *Klebsiella pneumoniae*) at the beginning and termination of a 24 hour period in which they had received 120 grams glucose per m^2 per 24 hours as the sole caloric source or 120 grams glucose plus 1.17 to 14.82 grams amino-nitrogen per m^2 per 24 hours. Each 24 hour balance period is demonstrated in Figure 2 by the circles. The linear regression line for these data has an r^2 of .6384 and may be described by $y = .4047X - 15.4545$. There was no significant difference in age or burn size among any of the groups.

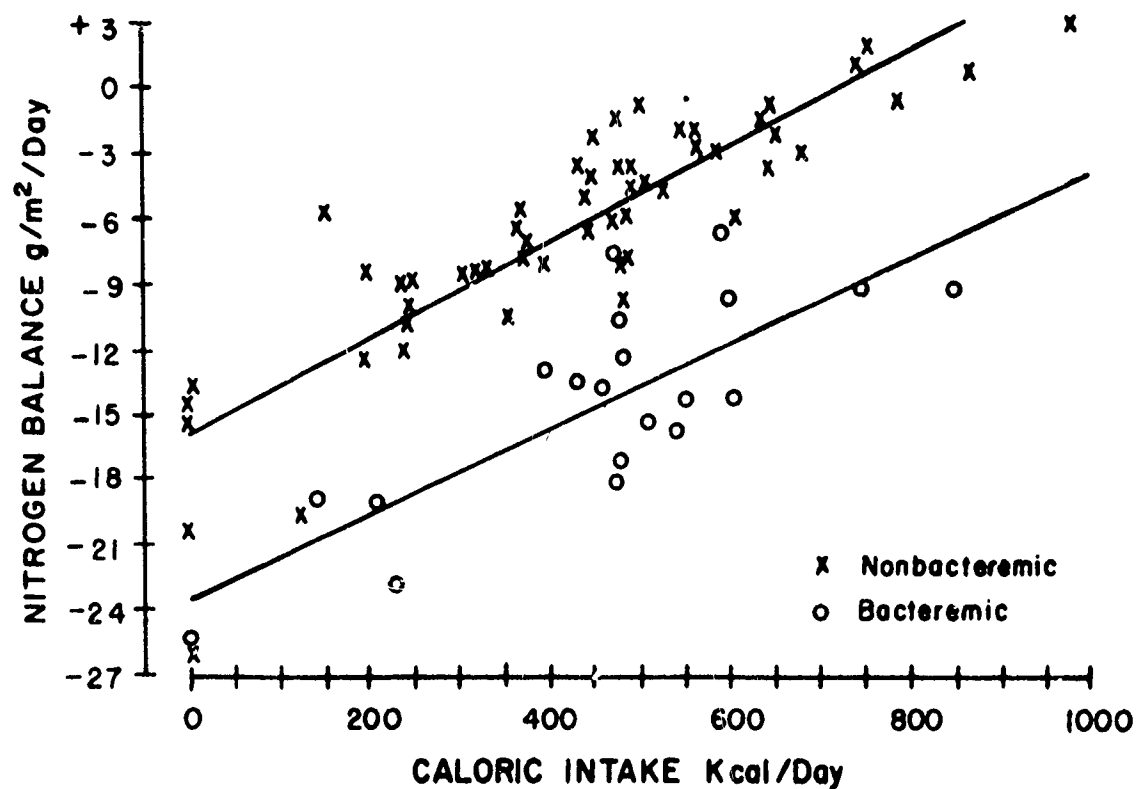
The slopes of the three regression lines in Figure 2 are not significantly different nor is there any significant difference between the nitrogen balance data for 60 gram glucose plus amino acids and 120 gram glucose plus amino acids. An analysis of covariance reveals that the regression line of the population receiving amino-acids is significantly different from both the regression line of the 60 gram glucose plus amino acid ($p < .001$) or 120 gram glucose plus amino acid ($p < .001$) groups.

The slopes of the regression lines for non-bacteremic and bacteremic patients receiving the same infusate are not significantly different. The populations represented by the regression lines which received the same infusates for bacteremic and non-bacteremic patients were significantly different: amino acid group, $p < .001$; 60 gram glucose plus amino acid group, $p < .001$ and 120 gram glucose plus amino acids, $p < .001$.

Figure 3 illustrates nitrogen balance as a function of caloric load irrespective of source for both non-bacteremic and bacteremic patients. The linear regression line for the non-bacteremic group has an r^2 of .7808 and may be defined by $y = .0221X - 16.0954$. The linear regression line for the bacteremic group ($r^2 = .6405$) is represented by $y = .0196X - 23.4426$. The slopes between the two lines are not significantly different but each line differs significantly from the other and represents a distinct population as determined by the analysis of covariance ($p < .001$).

Table 1 is a comparison of the 24 hour urinary data for 60 or 120 gram glucose plus amino acids versus glucose free amino acid infusates in bacteremic and non-bacteremic patients. Significant differences are seen in the non-bacteremic patients for the ratio of urine urea nitrogen to total urinary nitrogen, $p < .005$, the ratio of the quantity total

Figure 3. Nitrogen balance per square meter body surface corrected for alterations in blood urea nitrogen as a function of caloric intake irrespective of source for nonbacteremic and bacteremic patients.



urinary nitrogen minus urine urea nitrogen to nitrogen intake, $p < .001$, for per cent fractional sodium excretion, $p < .001$, and for elevation in blood urea nitrogen, $p < .01$. In the bacteremic patients, only the more pronounced elevation in blood urea nitrogen in the amino acid group differed significantly from the glucose plus amino acid patients ($p < .01$).

There were no significant differences in the 24 hour urinary data illustrated in Table I for the amino acid infusates between bacteremic and nonbacteremic patients. A comparison in the glucose plus amino acid groups between bacteremic and non-bacteremic patients demonstrates a significant difference for the fraction of excreted amino acids as a function of those infused, $p < .005$, UUN:TUN, $p < .05$, GFR, $p < .05$, percent fractional sodium excretion, $p < .05$ and change in plasma blood urea nitrogen, $p < .01$. Water balance and sodium and potassium loads were similar for the various infusates in both non-bacteremic as well as bacteremic patients.

Table II depicts the plasma values obtained for the various infusates for insulin, free fatty acids and glucagon in bacteremic and non-bacteremic patients. Significant differences are observed in non-bacteremic patients between those receiving amino acid infusates alone and those administered glucose plus amino acids for insulin, $p < .001$, free fatty acids, $p < .001$, glucagon, $p < .001$ and insulin to glucagon molar ratio, $p < .001$. Bacteremic patients demonstrated significant differences between the two types of infusions for insulin, $p < .001$, and free fatty acids, $p < .001$. A comparison between bacteremic and non-bacteremic patients receiving the same infusion reveals a significant difference for glucagon in patients receiving amino acid infusates free of glucose ($p < .001$) and insulin in patients receiving glucose plus amino acids, ($p < .05$).

The nitrogen balance may be expressed as a function of both the carbohydrate and amino-nitrogen input by forming a matrix. The nitrogen balance for a particular dose or combination of doses is calculated from the linear regression equations describing the data in Figures 1 and 2. Matrices for non-bacteremic and bacteremic patients are illustrated in Figures 4 and 5, respectively.

DISCUSSION

A hypermetabolic response occurs in both the uninfected and infected traumatized patient. Negative nitrogen balance, energy expenditure and weight loss are of far greater magnitude in the critically ill than in the uninjured chronically starved individual. Indeed, more than 200 grams protein may be catabolyzed per day to satisfy energy demands in the traumatized patient. Left unabated, the catabolic response results in excessive erosion of body mass, impaired organ function, an irreversible disruption of homeostasis and death. The provision of sufficient nitrogen and calories can reverse the process and thereby

TABLE 1. RENAL AND PLASMA DATA FOR NON-BACTEREMIC AND BACTEREMIC PATIENTS INFUSED WITH EITHER AMINO ACIDS OR GLUCOSE PLUS AMINO ACIDS.

	AMINO ACIDS	GLUCOSE & AMINO ACIDS	P
$\frac{TUN-UUN}{N_{in}}$			
Non-bacteremic	$0.419 \pm .181$	$0.125 \pm .099$	$< .001$
Bacteremic	$0.777 \pm .268$	$0.862 \pm .357$	N.S.
$\frac{UUN}{TUN}$			
Non-bacteremic	$0.705 \pm .060$	$0.811 \pm .090$	$< .005$
Bacteremic	$0.647 \pm .132$	$0.704 \pm .125$	N.S.
$\frac{GFR}{ml/min/m^2}$			
Non-bacteremic	66.7 ± 26.0	74.4 ± 31.5	N.S.
Bacteremic	56.2 ± 28.6	53.9 ± 21.2	N.S.
$\frac{NaFxEx}{\%}$			
Non-bacteremic	$0.670 \pm .194$	$0.142 \pm .012$	$< .001$
Bacteremic	$0.383 \pm .211$	$0.299 \pm .182$	N.S.
$\frac{KFxEx}{\%}$			
Non-bacteremic	22.01 ± 8.42	19.34 ± 7.07	N.S.
Bacteremic	29.37 ± 19.90	25.11 ± 8.81	N.S.
$\frac{\Delta BUN}{mg\% / m^2}$			
Non-bacteremic	$6.81 \pm$	$0.29 \pm$	
Bacteremic	$9.16 \pm$	$1.42 \pm$	$< .01$

TABLE II. PLASMA FREE FATTY ACIDS, INSULIN, GLUCAGON AND INSULIN TO GLUCAGON RATIO FOR NON-BACTEREMIC AND BACTEREMIC PATIENTS INFUSED WITH EITHER AMINO ACIDS OR GLUCOSE PLUS AMINO ACIDS.

	AMINO ACIDS	GLUCOSE & AMINO ACIDS	P
FREE FATTY ACIDS (mEq/l)			
Non-bacteremic	0.443 \pm 0.143	0.165 \pm 0.058	.001
Bacteremic	0.402 \pm 0.115	0.175 \pm 0.051	.001
INSULIN (uU/ml)			
Non-bacteremic	5.24 \pm 0.98	68.00 \pm 10.53	.001
Bacteremic	2.97 \pm 0.90	23.04 \pm 4.77	.001
GLUCAGON (pg/ml)			
Non-bacteremic	125.49 \pm 33.08	634.55 \pm 114.83	.001
Bacteremic	476.65 \pm 80.21	539.43 \pm 217.84	N.S.
INSULIN: GLUCAGON			
Non-bacteremic	.0267 \pm .0275	.2303 \pm .1925	.001
Bacteremic	.0087 \pm .0056	.0759 \pm .0815	N.S.

FIGURE 4. NITROGEN BALANCE AS A FUNCTION OF DOSE OF INTRAVENOUS INFUSION OF CARBOHYDRATE AND PROTEIN ADMINISTERED ISOSMOTICALLY IN NON-BACTEREMIC PATIENTS.

AMINO NITROGEN (grams)	GLUCOSE (grams)		
	0	60	120
0	-17.68	-10.71	-7.53
10	-10.45	-4.58	-1.71
15	-6.83	-1.51	-1.20

FIGURE 5. NITROGEN BALANCE AS A FUNCTION OF DOSE OF INTRAVENOUS INFUSION OF CARBOHYDRATE AND PROTEIN ADMINISTERED ISOSMOTICALLY IN BACTEREMIC PATIENTS.

AMINO NITROGEN (grams)	GLUCOSE (grams)		
	0	60	120
0	-24.00	-13.34	-15.45
10	-19.45	-10.52	-11.41
15	-17.18	-9.11	-9.38

achieve an anabolic state which is necessary for full functional recovery. Critically ill patients are often incapable of eating; however, positive nitrogen balance and satisfaction of energy demands have been achieved by intravenous administration of hyperoncotic glucose and protein infusates.⁴ These solutions which require administration^{5,6,7} through a central line, can serve as a source of systemic infection, can result in life threatening metabolic derangements⁸ and are impractical in the immediate post injury period when fluid balance, electrolyte composition and blood volume are being restored. Near isosmotic solutions which can be administered by peripheral vein obviate many of the hazards of the hyperoncotic infusates and are more practical when acute fluid, electrolyte and volume abnormalities require correction. With their use, the severe catabolic response is limited but often not corrected since satisfaction of energy requirements which may exceed 3000 calories per day, require unacceptably large quantities of fluid when nutrients are given in near isosmotic concentrations.

Glucose infusions in doses up to 100 grams per day provide a protein sparing effect which is proportional to the amount administered. Greater quantities result in very little additional reduction in nitrogen losses.⁹ Amino acid infusions free of glucose have also been shown to reduce proteolysis. Indeed, several reports indicate that a more substantial nitrogen sparing effect is achieved when amino acids are administered alone as compared to infusions of dextrose or dextrose in combination with amino acids.^{10,11} The proposed mechanism for this effect involves mobilization of endogenous fat stores as an alternative energy source. Infused dextrose causes an increase in circulating insulin which inhibits the release of free fatty acids from fat stores. The reasoning is that since sufficient quantities of free fatty acids are unavailable, energy demands are met by increased proteolysis. If dex-

4. Dudrick SJ, Wilmore DW, Vars HM and Rhoads JE: Long-term parenteral nutrition with growth, development, and positive nitrogen balance. *Surgery* 64:134, 1968.

5. Freeman JB, Lemire A and MacLean LD: Intravenous alimentation and septicemia. *Surg Gynec Obstet* 135:708, 1972.

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11. Blackburn GL, Flatt JP, Clowes, GHA, and O'Donnell TE: Peripheral intravenous feeding with isotonic amino acid solutions. *AM J Surg* 125:447, 1972.

trose is omitted from the infusate and only amino acids are given, insulin levels remain low and large quantities of free fatty acids are then released which provide the necessary energy substrate. Therefore, lesser amounts of endogenous protein need be catabolyzed to supply necessary energy demands.¹² Confirmation of these observations requires dose response data in a single population for both glucose and amino acid infusates. Unfortunately, these data have heretofore been unavailable. It should also be apparent that the most appropriate near isotonic infusate must not only provide superior nitrogen sparing when compared to other solutions but must also provide the necessary form of substrate for optimal metabolic activity and organ function.

Figure 1 illustrates dose response data for amino acid and amino acid plus glucose infusates. The validity of 24 hour dose schedules demonstrated in this figure was confirmed by obtaining almost identical values from patients who had received constant dose infusions spanning 3 to 9 days (Group III). These data indicate that calorie for calorie in a dose range appropriate for peripheral vein administration with acceptable fluid loads amino acids are no more effective than 60 grams glucose per m^2 plus amino acids in achieving nitrogen balance. Indeed, 60 grams protein per m^2 (9.6 grams nitrogen) gives the same nitrogen balance as 60 grams glucose per m^2 free of amino acids. Although 120 grams glucose per m^2 plus amino acids improves nitrogen balance over that achieved with 60 grams glucose per m^2 plus a comparable amino acid dose, it does so disproportionately less. Therefore, 60 grams glucose per m^2 plus amino acids achieves the greatest effect per gram.

Free fatty acid levels were significantly elevated and insulin concentrations significantly reduced in the amino acid infused patients when compared to those receiving amino acids plus glucose (Table II). The insulin and free fatty acid levels in the amino acid infused are comparable to those reported for patients who had received protein alone and were reported to demonstrate a superior nitrogen sparing effect.

In the face of elevated free fatty acids and decreased insulin levels in the amino acid group, we were unable to demonstrate any superiority in terms of nitrogen balance of amino acid infusates over glucose + amino acids. The exclusion of glucose from 2 dietary regimens on grounds of nitrogen sparing, therefore seems unwarranted.

The observation that there was no superiority of amino acid over glucose plus amino acid infusions may be explained by the fact that our nitrogen balances were corrected for alterations in the blood urea nitrogen. Indeed, the BUN rose 6 to 9 mg % per m^2 in the amino acid group compared to approximately 1 mg% in the glucose plus amino acid patients.

12. Blackburn GL, Flatt JP, Clowes GHA, O'Donnell TF and Hensle TE: Protein sparing therapy during periods of starvation with sepsis or trauma. Ann Surg 177:588, 1973.

($p < .01$). If not taken into account, what appears as improved nitrogen balance, may in actuality be retained urea. It may be most significant in patients who are administered glucose after having been maintained on protein. Under these circumstances, large amounts of urea will be excreted in the urine merely a reflection of more effective clearing of retained urea.

The balance studies were not corrected for the increased nitrogen loss which occurs from the burn wound. Wound nitrogen losses have been measured during the period of eschar separation and exposure of granulation tissue;¹³ however, as long as the eschar remains intact, as it did in all study periods, losses are minimal. Since patients from each of the study groups had comparable burn sizes and ages, the losses cancel themselves when the three groups are compared; i.e., there is no quantitative difference between the groups; however, all regression lines must be shifted the same distance in a negative direction if actual nitrogen balance for an individual patient is to be determined from the graphs.

Catabolism is more pronounced in bacteremic patients as illustrated in Figure 2. The regression line for the same infusate in bacteremic patients is significantly lower ($p < .001$) than in non-bacteremics. The response to amino acids alone is much poorer than when glucose plus amino-acids are given. Indeed, 60 grams glucose per m^2 alone is comparable to 125 grams protein (20 gm N) in terms of affecting similar nitrogen balances. Sixty grams glucose per m^2 plus amino acids provided the maximal nitrogen sparing effect since no significant difference was noted between 60 or 120 grams glucose/ m^2 plus amino acids. The matrixes provide a measure whereby the optimal utilization with the lowest dose of carbohydrate and amino acids may be determined. Both non-bacteremics and bacteremics achieve optimal effect per gram nutrient at a level of 60 grams glucose + 10 grams amino nitrogen/ m^2 /24 hours.

Although nitrogen balance is an important indicator of which type of infusate is more desirable, crucial to the success of any nutrient program is adequate provision of proper substrate for support of the metabolic demands of the organism. Chronically starved patients who derive their energy needs from free fatty acids improve their hepatic function significantly when dextrose is administered.¹⁴ Liver function as determined by indocyanine green clearance is impaired during infusion of amino acid solutions alone and may be restored to normal by adding glucose to the regimen. The effects are similar to those observed in patients who have gram negative septicemia; however, unlike the ab-

13. Corroff HS, Pearson E and Artz CP: An estimation of the nitrogen requirements for equilibrium in burn patients. Surg Gynec Obstet 112:159, 1961.

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normalities observed when amino acids are administered alone, hepatocyte function in septic patients cannot be corrected simply by administering glucose.¹⁵

Renal active transport processes for sodium and amino acid re-sorption are also impaired when amino acids serve as the sole exogenous energy source. In the face of similar sodium loads and sodium balances, fractional sodium excretion is diminished when glucose is added to the regimen. Thus addition of glucose results in more effective transport of sodium out of the filtrate. The kidney is better able to retain filtered amino acids as illustrated by the reduced fraction of infused amino acids excreted in the urine and the improved fraction of urea excreted to total nitrogen upon the addition of glucose to the infusate.

Total urinary nitrogen is comprised of ammonia, uric acid, creatinine, amino acids, peptides and urea nitrogen. Creatinine excretion, urinary pH and serum uric acid levels were similar in both the amino acid infused patients and those given amino acids plus glucose. Therefore, changes in TUN minus urea most likely reflect changes in the excretion of amino acids and peptides. Thus, TUN minus UUN divided by infused amino acids provides an estimate of the amount of amino acids lost expressed as a function of the amount infused. Also the ratio of UUN to TUN is a relative approximation of amino acids metabolized (Table I). Both ratios indicate more effective retention of amino acids and suggest greater incorporation into protein with lesser amounts degraded for gluconeogenesis when glucose is infused with the amino acids. Glucagon was elevated when compared to normal levels (75pg/ml) in the amino acid group in nonbacteremics but in bacteremics was markedly elevated and did not differ from values observed for patients infused with glucose plus amino acids. Glucagon stimulates hepatic glycogenolysis and gluconeogenesis, and therefore has been viewed as a "hormone of energy release."¹⁶

Insulin facilitates transport of amino acids into cells and hence their incorporation into protein. Glucose which accompanies the amino acids into the cells may well be necessary if the process of protein synthesis is to occur.¹⁷

In experimental animals glucagon is elevated after amino acid infusions and in normal subjects diminished following glucose

15. McDougal WS, Wilmore DW, Pruitt BA Jr.: Glucose dependent hepatic membrane transport in non-septic and septic thermally injured patients. Abstracts Society for Academic Surgery, 1976.

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administration.^{16,18} Thus, glucagon modulates the amount of energy available according to the specific needs of the organism. The elevated levels observed in the bacteremic and nonbacteremic glucose plus amino acid groups suggest that the stimulatory effect of amino acids in a catabolic patient overrides the suppressive effect of glucose and that glucagon may well be required for optimal amino acid utilization.

The molar ratios of insulin to glucagon in all groups is less than 1 and indicates that the glucose flow across the liver involves gluconeogenesis primarily from amino acid precursors. The larger ratios observed in the glucose plus amino acid groups is supportive of quantitatively lesser amino acid breakdown in this group when compared to patients receiving glucose free amino acid infusates.

The stress of bacteremia in the severely traumatized patient appears to be a powerful stimulus for glucagon production, since bacteremic patients receiving amino acids demonstrated exceedingly high levels. Since this is obviously an extreme energy demanding situation, the glucagon response is to be expected. The relatively low level, although elevated above normal, observed in non bacteremic patients receiving only amino acids would therefore appear to be less than that needed for optimum surgery utilization.

The efficacy of amino acid solutions administered alone is unsubstantiated in these studies since their infusion when compared to 60 grams glucose/m² plus amino acids given in equal caloric loads provides similar nitrogen balances in nonbacteremic patients and markedly inferior balances in the critically ill bacteremic patient. These observations were made in the face of exceedingly low insulin levels and markedly elevated free fatty acid concentration in the amino acid group. The administered amino acids are not utilized as well as an energy source when glucose is absent from the infusate as demonstrated by the reduced UUN:TUN ratio and the increased fraction of infused amino acids excreted in the urine. Indeed, the efficacy of low insulin levels is questionable since it is known that this hormone enhances transport of amino acids into cells and hence their incorporation into protein.¹⁶ The absence of glucose also impairs hepatic and renal transport processes.

PRESENTATIONS AND/OR PUBLICATIONS

None

18. Unger RH, Ohneda A, Aguilar-Parada E, and Eisentrout AM: The rate of aminogenic glucagon secretion in blood glucose homeostasis. JJ Clin Invest 48:810, 1969.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6954	76 10 01	DD-DR&E(AR)636	
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75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3A161102B71R		01		302	
b. CONTRIBUTING	61101A	3A16110A91C		00		076	
c. CONTRIBUTING							
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003500 Clinical Medicine							
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c. TYPE:				CURRENT			
d. KIND OF AWARD:							
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a US Army Institute of Surgical Research				NAME: ^a US Army Institute of Surgical Research			
ADDRESS: ^a Fort Sam Houston, Texas 78234				ADDRESS: ^a Burn Study Branch Fort Sam Houston, Texas 78234			
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NAME: Basil A. Pruitt, Jr, COL, MC				NAME: ^a Hugh D. Peterson, COL, MC			
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				SOCIAL SECURITY ACCOUNT NUMBER.			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
FOREIGN INTELLIGENCE NOT CONSIDERED				NAME: Barry A. Levine, MAJ, MC			
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Laser; (U) Bovie cautery; (U) Investing fascia; (U) Eschar; (U) Autograft; (U) Humans							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To evaluate the carbon dioxide laser for use in excision of full-thickness burns with attention towards time involved blood loss, and graft take involved in such excisions on burned soldiers.</p> <p>24. (U) Symmetrical excisions were performed on patients who were candidates for excision to the investing fascia. Laser excisions were compared to both scalpel and electrocautery excisions from the standpoint of blood loss and electrocautery excisions, time for excision, graft take and pre-and postop excision quantitative microbiology.</p> <p>25. (U) 75 07 - 76 09 The laser was found to be superior to scalpel excisions as far as blood loss, but was just as time consuming. The laser produced slightly less blood loss when compared to the electrocautery but took significantly longer time for the surgical procedure. The graft take in all three modalities was excellent. Because of the expense of the laser the surgical unwieldiness of the instrument and failure to demonstrate any significant difference from electrocautery, the laser in its present state was abandoned as an instrument for excision. If the machine can be made less cumbersome and less expensive, it should find a role in surgical excision. Sequential excision has been used to good effect in treatment of burns of the dorsum of the hand, especially those which are deep second-degree burns. Use of "meshed" graft to cover the excised wound bed results in maximum graft take and satisfactory long-term coverage and functional results.</p>							

^a Available to contractors upon originator's approval

TERMINATION

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: EXCISION OF ESCHAR IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

5 July 1975 - 30 September 1976

Investigators:

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE-SURGERY

REPORT TITLE: EXCISION OF ESCHAR IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam-Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Hugh D. Peterson, D.D.S., M.D., Colonel, MC
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Report Control Symbol MEDDH-288 (R1)

Studies prior to this reporting period demonstrated that the 100 watt carbon dioxide laser was not a practical instrument for excision to the investing fascia. The main disadvantages being its bulk and unwieldiness, plus cost.- It was in no way equivalent to the Bovie cautery except in excising large planar surfaces. With abandonment of the laser our attention has been focused more on the various modalities of excision and the refinement of patient selection criteria.

During this reporting period, 78 of 254 patients or 30% underwent excision procedure. These included excision to the investing fascia with immediate grafting, early sequential excision to viable tissue with delay in grafting, tangential excision with immediate grafting and delayed escharectomy.

Laser
Bovie cautery
Investing fascia
Sequential excision
Tangential excision
Eschar

EXCISION OF ESCHAR IN BURNED SOLDIERS

With abandonment of evaluation of the CO₂ laser, attention has been focused upon patient selection for excision and evaluation of the various techniques of excision. A total of 78 patients have undergone some form of excision during this reporting period.

The criteria for excision at the US Army Institute of Surgical Research are fairly well established. There are three categories of patients, the first category is small burns either full thickness or deep second degree injuries that can be excised in their entirety and grafted immediately. This results in a closed wound that heals within two weeks, and greatly decreased morbidity of small nonlife threatening burns. The second category is burns in the 30 to 70% size that have a significant area or areas of full thickness or very deep partial thickness that lend themselves to excision. This does not decrease hospital time but removes areas prone to burn wound problems. This is especially true if the legs or perineum are involved. A subcategory is burns of this size that have special areas that will benefit from excision by way of earlier return to function and a better final skin coverage. At our unit this is largely limited to the hands. The final category is burns greater than 70% with a large portion of full thickness injury. Historically these have done very poorly and conservative management with a topical agent offers no improvement in mortality, no matter what agent is used. In this final category some form of excision procedure seems at this time to be the only rational approach to attempting to increase survival.

The various types of excision being evaluated are, excision to the investing fascia with either immediate isografting or coverage with a biologic dressing, tangential excision and grafting, sequential excision to viable tissue and grafting, and lastly escharectomy either early or late.

Twenty-five patients were treated with excision to the investing fascia. There was a total of 35 procedures accomplished in these 25 patients. Seventeen patients were in the 40 to 70% burn size and there were six deaths in this group. This single figure means very little since it must be borne in mind that the patients subjected to an excision are those that have the largest areas of full thickness injury. There were a total of eight patients with burns greater than 70% that underwent formal excision with no survivors. In general the plan is to excise the patient as soon as they are hemodynamically stable from their resuscitation and when necessary to stage the excision so that never more than two extremities are done at one time. The excisions that do the best are those that can be isografted on fascia at the time of excision. It is of interest to note that all of the deaths in 40 to 70% burn size were at the upper end of that category and did not have sufficient donor sites to allow initial isografting. All the nonsurvivors in that group were subjected to the problems of repeated homograft changes. In the

burns greater than 70% none of them had available donor areas so that more than a small portion of the initial excision could be isografted. Both of these demonstrate the great difficulty of wound coverage in large excisions.

Tangential hand excisions have been carried out at the US Army Institute of Surgical Research for the past two and a half years. They follow the classic principles of tangential excision and immediate grafting and are limited to deep partial thickness injuries and full thickness injuries where there are no obvious deep structures involved. The hands are isografted immediately in all instances and when the criteria for patient selection are rigidly adhered to functional return is complete. The hands are excised as soon as the patient is hemodynamically stable post resuscitation and the procedure is not done in patients with burns in excess of 60% unless it is done in concurrence with some other form of excision requiring a general anesthetic. During this reporting period twenty three patients underwent tangential excision of the hands with a total of 34 hands being excised. There were five deaths in that age group, all in large burns that underwent hand excision concomitantly with other excision procedures. In the surviving patients those that had strict adherence to selection criteria demonstrated a complete return of function while those where the criteria were extended had various extensor defects.

Sequential excision to viable tissue with delayed grafting, more properly termed escharectomies, were carried out in 27 patients with a total of 31 procedures. The escharectomies applied in two instances. Early in the postburn course to rid the patient of eschar and start an earlier preparation of the wound for definitive grafting. Eleven patients were treated in this fashion early in the postburn course and there were seven deaths. These were all in burns greater than 60%. The early escharectomy was employed either because the burn wound did not lend itself to formal excision to the investing fascia, i.e. perineums, buttocks or other difficult areas to excise to the fascia. The higher incidence of mortality in like size burns would suggest that early escharectomy is not tolerated as well as excision to the investing fascia with coverage with a biologic dressing. The second instance in which escharectomy is performed is later in the postburn course to remove tenacious eschar. This is done in the third to fourth week postburn, 16 patients were subjected to this treatment during the last reporting period with no mortality. This is an excellent technique to remove tenacious eschar and hasten the time to grafting. We have been successful with the delayed escharectomy using either biologic dressings in the form of allograft or xenograft or simply coarse mesh gauze as sulfa soaks.

Excision in the truly large burns, those exceeding 65 to 70% is certainly a time consuming and difficult procedure, but with the

complete failure of topical agents or conservative measures to improve mortality in burns that size or larger it appears evident that some excision modality is at this time the only rational approach to improving survival.

PUBLICATIONS AND/OR PRESENTATIONS:

Levine NS, Salisbury RE, Peterson HD, Pruitt BA Jr. Clinical Evaluation of the Carbon Dioxide Laser for Burn Wound Excision: A Comparison of the Laser, Scalpel, and Electrocautery. J Trauma 15: 800-807, 1975.

Levine NS, Peterson HD, Salisbury RE, Pruitt BA Jr: Laser, Scalpel, Electrosurgical, and Tangential Excisions of the Third Degree Burns: A Preliminary Report. J Plast Reconstr Surg 56:286-296, 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY					1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL
					DA OD 6978	76 10 01	DD-DR&E(AR)636
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DESIG INSTR ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUMMARY
75 07 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3S1611102BS05		00		081	
b. CONTRIBUTING	61102A	3A161102B71R		01		184	
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Evaluation of Synthetic Sheeting as Operating Room Drape Material For Use in a Military Burn Unit (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
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17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
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20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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21. GENERAL USE				ASSOCIATE INVESTIGATORS			
FOREIGN INTELLIGENCE NOT CONSIDERED				NAME: Robert B. Lindberg, PhD			
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military burn unit; (U) Operating room based infections; (U) Surgical drapes; (U) Surgical gowns							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number Precede text of each with Security Classification Code)							
<p>23. (U) Evaluation in terms of draping characteristics, absorbency, physician acceptance, and bacterial barrier qualities of a Spunbonded Olefin-cellulosic Laminated sheeting as surgical drapes and gowns. A decrease in bacterial seeding of operative wounds via drapes will minimize postoperative wound infections decreasing subsequent morbidity and mortality in injured troops.</p> <p>24. (U) Laboratory assessment of bacterial barrier of synthetic sheeting. Clinical use of drapes on burn patients to determine surgeon acceptability. Photographic documentation of draping characteristics, absorbency, and "run-off". Pre- and postoperative cultures at margin of operative field. Temperature monitoring to, determine heat transmission characteristics.</p> <p>25. (U) 75 07 - 76 09 Bacterial barrier properties of drape materials have been assessed and the results analyzed. Differences in penetration exist between organisms and between drapes but interaction is also significant. Overall penetration was 12-30% of test sites and differences between drapes are not great suggesting an inadequate barrier function of all samples. Reduction of sheeting pore size or "tighter" weave of material appear necessary to prevent passage of bacteria.</p>							

^a Available to contractors upon originator's approval

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AND 1498 1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3S1611102BS05, MILITARY BURN RESEARCH

**REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING
ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN
UNIT**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Basil A. Pruitt, Jr., MD, FACS, Colonel, MC
Robert B. Lindberg, PhD
Arthur D. Mason, Jr., MD**

Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

PROJECT NO. 3S1611102BS05, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING
ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN
UNIT

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Basil A. Pruitt, Jr., MD, FACS, Colonel, MC
Robert B. Lindberg, PhD
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

The bacterial barrier properties of six coded samples of synthetic surgical drape material have been assessed. The penetration at 42 sites of each drape material by inocula of five organisms, Pseudomonas, Serratia, Klebsiella, E. coli and Staphylococcus, has been assessed using a test system developed at this Institute.

Penetration rates between drapes were significant only for Klebsiella, E. coli and Staphylococcus, but the variation within drapes was also significant, indicating that the variation may be either in the drape samples or the evaluation technique. No consistently "best" drape was identified. Mean penetration of drape samples by organisms ranged from 18 to 37%, with the penetration by Pseudomonas significantly higher than that for other organisms. Penetration of drape samples by the three organisms showing significant differences in penetration rates varied from 12 to 30%, indicating that all of the tested samples were imperfect microbial barriers and were penetrated too readily to afford significant protection. The findings suggest that two drape samples showed slightly better barrier properties and that any physical or processing characteristics common to those two samples should be the subject of further study and evaluation.

Military burn unit
Operating room-based infections
Surgical drapes
Surgical gowns

EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

Commonly used surgical drapes are imperfect microbial barriers and as such may inadequately protect the operative wound of combat-injured soldiers from contamination by organisms from adjacent skin areas or the environment. Drapes made from synthetic fibers generally have poor draping characteristics, do not transmit water vapor and permit quantitative run-off of liquids. Earlier evaluations indicated that sheeting made from Spun-bonded Olefin possessed excellent microbial barrier properties and transmitted water vapor but not liquid water, but had marginal draping characteristics and still permitted liquid run-off. In an attempt to both minimize liquid run-off and soften the material, a single layer of Spun-bonded Olefin was then sandwiched between two layers of absorbent cellulosic fibers. Such sheeting was better accepted clinically but the laminating procedure compromised the barrier properties of the sheeting and permitted the irregular passage of test bacteria when microbial transmissivity was assessed using laboratory techniques developed at this Institute. Spun-bonded Olefin sheeting of varied thickness, produced by various fabrication procedures, is being evaluated to define those characteristics of a clinically acceptable surgical drape material which will provide a reliable bacterial barrier.

Methods

Two and one-half inch discs were cut from six coded samples of synthetic sheeting and sterilized in glass Petri dishes using ethylene oxide. Test strains of Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Serratia marcescens collected from clinical microbiology specimens were used as the inocula. Cultures of these organisms stored in suspensions of sterile milk at -70° , thawed and grown for 18 hours in trypticase soy broth, constituted the inoculum.

The sterile discs of sheeting were placed aseptically on fresh blood agar plates. Six drops of each test culture were placed on each disc, spaced equidistant at a distance of one cm. from the edge of the disc of sheeting material. The drops were left in place for four hours at room temperature. If the drops of inoculum did not spread and were still evident at the end of the four-hour exposure time, the remaining fluid was meticulously removed by aspiration with a sterile capillary pipette. The sheeting discs were then removed from the culture plates and the plates incubated at 37°C for 24 hours, following which the plates were examined for evidence of growth at each site of inoculum drop placement. Seven replicates were run for each test organism so that penetration of 42 individual inoculum drops could be assayed.

Results

The fraction and percentage of penetration of inoculum sites of each sheeting sample by each of the test organisms is shown in the table. Statistical assay of these results revealed no significant difference in penetration by Pseudomonas and Serratia organisms between drapes, but did reveal significant differences of penetration of the drape materials by the other three test organisms, Klebsiella, E. coli, and Staphylococcus. For the latter three organisms, the difference in drape penetration was significant at the one per cent level, but it should be noted that the interaction was also significant at the one per cent level.

Discussion

Chi square analysis of the test organism penetration of the six coded surgical sheeting samples revealed no systematic difference among samples based on sample code. The differences among drapes and among organisms were found to be significant but the interaction was also significant, necessitating analysis of drape penetration by individual organisms. Significant differences existed between drape penetration by Klebsiella, E. coli and Staphylococcus, but not by Pseudomonas or Serratia. The chi square within drapes was significant or nearly significant for all organisms, suggesting some real variation in either the drape samples or the evaluation technique.

Counting of positive penetration has been conservative, i.e., if a single area of bacterial colonies touches two sample sites, both were considered to have shown penetration, but it was possible that penetration had occurred at only one site with spreading of the colonies. Such a counting procedure may increase the variation in penetration observed beyond an expected value.

If the results of the Pseudomonas and Serratia testing are excluded, the interaction term remained significant and there was no difference between penetration by the remaining three organisms, i.e., Klebsiella, E. coli and Staphylococcus. The penetrations of each of these three individual organisms showed significant differences among drapes but the sampling technique itself, as previously noted, showed wide variation. Among those organisms where the difference in penetration between drapes was significant, Friedman two-way nonparametric analysis suggested there to be no consistently "best" drape. That analysis did suggest that if samples # 298-132-1 and #298-132-4, possess a common property imparted by either thickness or fabrication processing, further studies of that property should be made.

Mean penetration of drape samples by organisms range from 17.5% for Serratia to 36.5% by Pseudomonas organisms, with only the penetration rate by Pseudomonas being different from the other organisms. Determination of mean penetration by each drape material revealed lower rates of penetration of the two drape materials previously noted, both in terms of penetration by all organisms and penetration by Klebsiella, E. coli and Staphylococcus.

Organism: Sample Code:	Klebsiella sp		E coli		Ps aeruginosa		Serratia		S aureus		Totals	
	Pos	%	Pos	%	Pos	%	Pos	%	Pos	%	Pos	%
298-132-1	7/42	16.7	5/42	11.9	10/42	23.8	6/42	14.3	3/42	7.1	31/210	14.7
298-132-2	17/42	40.5	6/42	14.3	15/42	35.7	6/42	14.3	5/42	14.3	50/210	23.8
298-132-3	8/42	19.1	12/42	28.6	18/42	42.9	6/42	14.3	6/42	14.3	50/210	23.8
298-132-4	1/42	2.4	3/42	7.1	11/42	26.2	5/42	11.9	14/42	33.3	34/210	16.2
298-132-5	9/42	21.4	15/42	35.7	19/42	45.2	10/42	23.8	11/42	26.2	64/210	30.5
298-132-6	10/42	23.8	19/42	45.2	19/42	45.2	11/42	26.2	8/42	19.1	67/210	31.9

Over-all penetration occurred in 12-30 % of test sites and the differences between drapes were not great, indicating that all the tested samples were penetrated too readily by the test organisms and are, in essence, inadequate microbial barriers. The findings suggest that a "tighter" sheeting is required, especially if organisms like Pseudomonas are to be excluded from passage in the presently employed test system.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY					1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
					DA OD 6976	76 10 01	DD-DR&E(AR)636	
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75 07 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		A. WORK UNIT
10. NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER
A. PRIMARY		61101A		3A161101A91C		00		083
B. CONTRIBUTING								
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11. TITLE (Precede with Security Classification Code) ^a (U) Studies of the Effect of Variations of Temperature and Humidity on Energy Demands of the Burned Soldier in a Controlled Metabolic Room (44)								
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a								
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19. RESPONSIBLE DOD ORGANIZATION					20. PERFORMING ORGANIZATION			
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21. GENERAL USE					ASSOCIATE INVESTIGATORS			
FOREIGN INTELLIGENCE NOT CONSIDERED					NAME Arthur L. Mason, Jr, MD			
					NAME Basil A. Pruitt, Jr, COL, MC DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Metabolism; (U) Heat loss; (U) Evaporative water loss; (U) Controlled environment; (U) Humans; (U) Critical temperature; (U) Temperature								
23. TECHNICAL OBJECTIVE ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)								
<p>23. (U) To define the relationship between surface cooling and hypermetabolism in a controlled ambient environment, to determine the mediator of the profound hyper-catabolic response following thermal injury, and the mechanisms of stress-induced heat production in burned soldiers.</p> <p>24. (U) The use of a controlled environmental study room to measure metabolic rate at various temperatures; concomitantly, measurements of water loss, heat production, core temperature, mean skin temperature, and calculation of heat transfer coefficients and routes of heat loss. Simultaneously, measurements of blood substrate, urine and plasma catecholamines, blood hormone levels, and correlation of total body metabolism with energy demands are performed.</p> <p>25. (U) 75 07 - 76 09 Prostaglandin levels in the blood of burn patients were normal and the burn wound did not appear to be a site of synthesis which contributed to system levels. Serum from burn patients resulted in a febrile response when injected into the thermoregulatory area of rabbits, and the mediator substance is thought to be endogenous pyrogen. T_4, the active thyroid hormone, is low following injury, and T_4 degradation appears to be toward alternate pathways, resulting in reversed T_4 synthesis. The significance of this chemical hypothyroidism is unknown. Glucose flow unimpaired by injury per se is diminished by supervening sepsis. Infusion of glucagon increases core temperature and metabolic rate in septic patients and increases hepatic gluconeogenesis.</p>								

^aAvailable to contractors upon approval of sponsor's approval

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PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. THE FORMS TAPRA 100100 AND 1498 100100 ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: STUDIES OF THE EFFECT OF VARIATIONS OF TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Douglas W. Wilmore, MD
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Louis H. Aulick, PhD, Major, MSC
Basil A. Pruitt, Jr., MD, Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: STUDIES OF THE EFFECT OF VARIATIONS OF TEMPERATURE
AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER
IN A CONTROLLED METABOLIC ROOM

US Army Institute of Surgical Research, Brooke Army Medical Center,
Port Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Douglas W. Wilmore, MD
Arthur D. Mason, JR., MD
Louis H. Aulick, PhD, Major, MSC
Basil A. Pruitt, Jr., Colonel, MD

Reports Control Symbol MEDDH-288(R1)

To determine the afferent mediator of the hypermetabolic response to injury, blood samples from thermally injured patients were analyzed for prostaglandin A, E, and F. Serum concentrations were unchanged from normals and femoral vein concentrations were similar in patients with and without leg injuries. Injection of serum from burn patients into the preoptic area of rabbits elicited a febrile response in 9 of 13 samples tested while serum from 6 normals evoked no response. The factor in the serum from burn patients was heat labile and limulus-lysate negative, suggesting the pyrogenic material is endogenous pyrogen.

Studies in 5 burn patients demonstrate the presence of clinical hypothyroidism following injury; there is a fall in serum T_3 and a rise in the attenuated component, reversed- T_3 . The significance of this hypothyroidism is yet to be determined.

Endogenous pyrogen
Prostaglandin
Thyroid hormones

STUDIES OF THE EFFECT OF VARIATIONS OF TEMPERATURE AND HUMIDITY
ON ENERGY DEMANDS OF THE BURNED SOLDIER IN
A CONTROLLED METABOLIC ROOM

Burn patients are hypermetabolic and hyperthermic. At any ambient temperature studied, core and mean skin temperatures in thermally injured patients are elevated 1-2° above temperatures observed in normal individuals (1). When allowed to adjust ambient temperature, burn patients select a warmer environment than normals to achieve comfort, in spite of the fact that the patients have elevated core and mean skin temperatures (2). The ambient temperature selected is generally related to burn size. Thermally injured patients may shiver in thermal neutral or warm environments (29-32°C), and patients with extensive thermal injuries rarely sweat, even in hot environments (>35°C). This evidence has been interpreted to suggest that thermal injury results in an elevation of the central reference temperature, and this apparent upward shift in setpoint activates the sympathetic nervous system which in turn raises body temperature. Fever secondary to infection is the result of two distinct physiologic mechanisms: vasoconstriction which prevents heat loss from the body and hypermetabolism which increases heat production.

The burn wound is highly vascularized and surface blood flow is increased; core-skin heat transfer increases with burn size (3), and leg blood flow is largely dependent on the extent of surface injury to the extremity (4). Because mechanisms for vasoconstriction and core insulation are not normal, the burn patient must rely on hypermetabolism as the primary mechanism for increasing body temperature.

The cause of the upward reset in the reference temperature in burn patients is unknown. Blocking afferent nervous input to the brain by topical anesthesia or applying topical anesthesia to the

1. Wilmore DW, Long JA, Skreen R, Mason AD Jr, Pruitt BA Jr: Catecholamines: mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653-668, 1974.

2. Wilmore DW, Orcutt TW, Mason AD Jr, Pruitt BA Jr: Alterations in hypothalamic function following thermal injury. *J Trauma* 15:697-703, 1975.

3. Wilmore DW, Mason AD Jr, Johnson DW, Pruitt BA Jr: Effect of ambient temperature on heat production and heat loss in burn patients. *J Appl Physiol* 38:593-597, 1975.

4. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on peripheral circulation in thermally injured patients. In press, *J Appl Physiol*.

burn wound has not reduced the hypermetabolic response (5). A single patient studied with spinal cord transection was markedly hypermetabolic in spite of almost complete denervation of the burn wound. However, the brain appears essential for the post-traumatic metabolic response, and hypermetabolism was observed in a burn patient with brain death.

Post-traumatic hypermetabolism is also attenuated during morphine anesthesia (6). This evidence suggests that afferent nervous input to the brain is not the cause of the hypermetabolic response following injury but that the brain (hypothalamus) is necessary to receive the signal and to integrate the final neurohormonal response. This report evaluates factors which are transmitted in the blood and could initiate the upward shift in central reference temperature, and evaluates new evidence which demonstrates further interaction between the sympathetic nervous system and metabolism of thyroid hormone.

Methods

Arterial and venous blood samples were obtained between the third and 31st postburn day from six burn patients (mean age 30 years, mean burn size 43 per cent), the plasma immediately separated, indomethacin added and the aspirates frozen. Prostaglandin A, E, and F were analyzed, using specific antibody techniques, and the results compared with blood obtained from normal controls. In addition, arterial venous samples were drawn simultaneously from 15 patients (mean age 30 years, mean burn size 45 per cent total body surface), with burned and unburned lower extremities to determine the contribution of the injury to the prostaglandin levels.

Heparinized venous blood was collected from burn patients and normals in pyrogenic-free syringes, the serum separated and transferred to pyrogen-free vials which were heat sealed. The samples were frozen until analysis, when they were thawed, the vial opened using sterile technique, and a micro aliquot injected through indwelling chronic cannula in rabbits, placed by standard stereotatic technique so the

5. Wilmore DW: Hormonal Responses and their Effects on Metabolism. Surgical Clinics of North America, edited by G. A. Clowes, Jr. (In press).

6. Taylor JW, Hander EW, Skreen R, Wilmore DW: Effect of CNS narcosis on the sympathetic response to stress. J Surg Research 20: 313, 1976.

distal tip lies in the preoptic area of the hypothalamus. Rectal temperature was monitored over the next two hours. Serum that yielded a febrile response was heat-treated and re-evaluated, and also tested by limulus lysate assay for endotoxin.

Serial plasma samples were obtained from five male thermally injured patients (mean age 29 years, mean burn size 66.5 per cent TBS) and evaluated by radioimmunoassay techniques for T_4 , T_3 , reverse T_3 and TSH.

RESULTS

Concentrations of prostaglandin A, E, and F were similar to those observed in normals (Table 1), and did not attain levels similar to those reported in pregnant women. All arterial concentrations appeared slightly lower than venous levels, presumably because the lung activity degrades these components. The burn wound did not contribute to the production of PGE or F; blood obtained from patients with and without leg burns showed similar concentrations in the femoral vein. Absolute prostaglandin levels were unrelated to burn size and did not change with time postburn.

Injection of normal serum into the preoptic area of the rabbit resulted in a 0.1°C rise in rectal temperature in 6/6 control subjects. Serum from 9/13 burn patients elicited a febrile response (0.63 - 0.93°C) following hypothalamic injection. Limulus lysate assay was negative in all these samples, and, after heat treatment of the serum, the response was attenuated, suggesting endogenous pyrogen mediated the thermogenic response.

T_4 levels were below normal levels in the burn patient, but the free thyroid index was normal, suggesting that T_4 concentrations were within low physiologic ranges and the low concentrations resulted from the low serum proteins which are observed following thermal injury. In contrast, triiodothyronine (T_3), the active hormone, was abnormally low throughout the first two weeks of injury (Table 2), and returned to normal with recovery. In contrast, there was an early rise in reverse T_3 which returned to normal levels during the second week postinjury. Further studies during septic episodes in burn patients two to three weeks postinjury demonstrate that the marked rise in reverse T_3 ($>125\text{ ng/dl}$) is associated with this complication; T_3 is further suppressed. Despite the depressed T_3 and low T_4 values, basal TSH values are $<10\text{ } \mu\text{IV/ml}$, suggesting alterations in the hypothalamus-pituitary-thyroid axis.

Table 1. Prostaglandin Concentrations (PG/ml Plasma)
(Mean \pm S.E.)

Subjects	No. Samples	No. of Samples	Sample Site	PGE	PGF	PGA
Burn patients (3-31 days)	6	25	Arterial	990 \pm 90	360 \pm 70	1600 \pm 90
Burn patients (6-21 days)	6	14	Arterial	950 \pm 120	410 \pm 120	1590 \pm 120
Normals	4	4	Venous	1020 \pm 290	100 \pm 10	1520 \pm 80
Pregnant women*	6	6	Venous	2680 \pm 480	490 \pm 130	3220 \pm 390
Pregnant women*	6	6	Uterine vein	2530 \pm 580	440 \pm 130	2670 \pm 450
Burn patients	15	15	Arterial	850 \pm 134	281 \pm 114	—
			Femoral vein	974 \pm 140	375 \pm 234	—
Burn patients (no leg burn)	4	4	Arterial	713 \pm 160	251 \pm 157	—
			Femoral vein	889 \pm 230	356 \pm 167	—
Burn patients (leg burns)	11	11	Arterial	838 \pm 177	200 \pm 101	—
			Femoral vein	1005 \pm 177	382 \pm 261	—

*From the data of Becker, R. A. and Sperdyff, L.

Table 2. Thyroid Studies in Burn Patients

Postburn Day	T ₄ (μg%)	FTI	T ₃ (ng%)	RT ₃ (ng%)	TSH (μ IU/ml)
3	2.5	3.8	52	94	4.1
6	2.5	3.0	26	104	2.2
8	3.7	6.1	36	76	4.2
10	5.1	6.2	31	85	3.2
13	3.8	5.0	16	56	3.7
15	5.0	6.3	44	66	4.7
Normal Range	4.5-11.5	3.8-13.4	80-180	36-84	10

DISCUSSION

Prostaglandin synthesis in tissues is stimulated by hypoxia, resulting in the elaboration of PGE, a vasodilator which antagonizes the constrictive effect of catecholamines to increase blood flow to the anoxic tissue. PGE (but none of the other prostaglandins) injected into the hypothalamus causes a febrile response (7). It is appealing to speculate that prostaglandins serve as mediators for local dilatation of the wound and also stimulate the alteration in central reference temperature. This hypothesis was not confirmed by the data. Because prostaglandins are rapidly degraded at the tissue level, these present analytical techniques may not be sensitive enough to detect increased prostaglandin activity and further analysis of PGE metabolites is now being planned. However, these prostaglandins are increased in pregnant women, and these patients do not exhibit alterations in the thermoregulatory mechanisms.

A febrile response was observed when serum from burn patients was injected into the temperature regulation center of the rabbit. This substance is heat labile and thought to be endogenous pyrogen, the biochemical product of lymphocytes and other phagocytic cells. Previous attempts to detect endogenous pyrogen in man during bacterial or viral infection have not yielded such a high percentage of febrile responses, suggesting that the inflammatory responses in the burn wound may continually liberate this mediator.

Whether endogenous pyrogen is in fact the mediator of the metabolic response to thermal injury, or just one of a number of factors which mediate post-traumatic events, is not known. Animal studies are in progress to evaluate the effect of the white cell in eliciting the metabolic and hyperpyrexia response to injury.

Burn patients appear chemically hypothyroid, although metabolic rate is elevated because of increasing discharge of the sympathetic nervous system. A fall in T_3 has been associated with increased gluconeogenesis in man, which apparently alters T_4 conversion to T_3 in the liver. Reversed T_3 , an iodinated compound with low biological activity, appears to be generated, although the exact site of synthesis of this substance is not known. The effect of this hypothyroidism on cell and organ function in injured man is now being evaluated in an animal model.

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PRESENTATIONS

Becker RA: Depressed serum trifodothyronine (T_3) levels following thermal injury. 60th Annual Meeting of the Federation of American Society for Experimental Biology, Anaheim, California, 10 April 1976.

PUBLICATIONS

Becker RA, Johnson DW, Woeber KA, Wilmore DW: Depressed serum trifodothyronine (T_3) levels following thermal injury. Fed Proc 35: 216, 1976.

RES 4 AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY 76 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY ^a U	6. WORK SECURITY ^a U	7. REGRADING ^a NA	8A. DISSEM INSTR ^a NL	8B. SPECIFIC DATA- CONTRACTOR ACCESS <input type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61101A	3A161101A91C		00		079	
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11. TITLE (Precede with Security Classification Code) ^a (U) Development and Analysis of an Animal Model for the Post Thermal Injury Hypermetabolic Response Found in the Burned Soldier (44)							
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RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: David N. Herndon, CPT, MC			
TELEPHONE: 512-221-2720				TELEPHONE 512-221-4440			
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22. KEYWORDS (Precede EACH with Security Classification Code) (U) Oxygen consumption; (U) Hypermetabolism; (U) Thermal injury; (U) Thyroid; (U) Catecholamines							
23. (U) To develop a reliable and easily reproducible small animal model of the postburn hypermetabolic response of humans; to better delineate the endocrine mediators of this response; to investigate the afferent mechanisms that initiate this response; to investigate the response of isolated tissues to determine which organs are hypermetabolic and which are normal or hypometabolic postburn; to better delineate the cellular mechanisms of thermogenesis in the postburn hypermetabolic state.							
24. (U) Approximately 50 animals each will be distributed as follows: (a) snam anes- thetized it (b) burned on 60% TBS with standard scald; in each of the following treatment groups: (1) thyroidectomized one month prior to study, (2) adrenalectomized one month prior to study, (3) irradiated 2 wks prior to study to produce leukopenia; (4) treated with B.I.D. injections of 1 mgm/kgm indomethacin starting 2 days prior to start of study drug to continue through study period. (5) treated with 5 different topical anti- microbials, (6) treated with varying diet, (7) infected with topical pseudomonas. These groups will be prepared in rats mature but growing (180 gm) rats at their plateau growth phase (approx. 540 gm) guinea pigs mature but growing (approx. 540 gm) and guinea pigs at plateau growth phase (i.e. 1000gm). Whole body oxygen consumption will be determined in modified Warburg apparatus on each day from seven days prior to start of study to the time of burned animals complete wound healing. In vitro tissue oxygen lactate glucose and glycogen consumption or production of liver slices, diaphragm sections and white fat cell suspensions will be made from all treatment groups at varying periods in the postburn hypermetabolic response and compared with matched control.							
25. (U) 76 01 - 76 09 A reproducible rat and guinea pig model demonstrating 40 to 30% increases in metabolic rate post 60 and 40% burns respectively, relative to matched controls. All studies at thermal neutral (32°C) have been established p<.0001, for each group studied.							

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ANNUAL PROGRESS REPORT

**PROJECT NO: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH**

**REPORT TITLE: DEVELOPMENT AND ANALYSIS OF AN ANIMAL MODEL FOR
THE POST THERMAL INJURY HYPERMETABOLIC RESPONSE
FOUND IN BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**David N. Herndon, MD, Cpt, MC
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Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: DEVELOPMENT AND ANALYSIS OF AN ANIMAL MODEL FOR THE POST THERMAL INJURY HYPERMETABOLIC RESPONSE FOUND IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1976-30 September 1976

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A reproducible small animal model simulating the post thermal injury hypermetabolic response of humans was developed. The oxygen consumption of one hundred 60% burned rats was approximately 1.33 times that of a group of 100 weight-paired controls from the 5th through the 45th post burn day when the animals were studied at 27°C and 32°C ($p < 0.0001$ at both temperatures). The metabolic rates of normal and burned rats were increased at 27°C relative to their metabolic rates at 32°C. These increases were proportional in each group. A group of 20 guinea pigs with 40% burns, 70 guinea pigs with 60% burns and 110 weight-paired controls gave similar results. The ratios of their observed to predicted normal metabolic rates were 1.33, 1.39, and 1.00 respectively at 32°C ($p < 0.0001$). The metabolic rates of burned mature rats were also elevated over those of weight-paired controls. The oxygen consumption of a group of forty 60% burned, 540 gm rats was 1.35 times that of weight-paired controls. The mortality of a 60% burn in these mature rats was 90% by post burn day 30, whereas it was only 15% in 180 gm rats burned during their linear growth phase (180-500 gm). When compared with 60 intact normal rats from the 7th to the 25th post burn day, the ratio of observed/predicted oxygen consumption of thyroidectomized rats was 0.61, that of 20 thyroidectomized burned rats was 1.00, and that of 60 normal burned rats was increased to 1.41 ($p < 0.01$).

Oxygen consumption
Hypermetabolism
Thermal injury
Thyroid
Catecholamines

DEVELOPMENT AND ANALYSIS OF AN ANIMAL MODEL FOR THE POST THERMAL INJURY HYPERMETABOLIC RESPONSE FOUND IN BURNED SOLDIERS

Hypermetabolism is a salient feature of the human response to thermal injury. Recent studies by Wilmore, et al¹ have shown that the resting metabolic rate increases with burn size in a curvilinear fashion (approaching 70 to 75 Kcal/M²/hr as burn size exceeds 50% of total body surface).² This is approximately twice the normal metabolic rate. Other disease states also stimulate hypermetabolism; peritonitis can cause a 25% increase in energy consumption, a multiple trauma patient on a respirator can have up to a 75% increase, but even this is only equivalent to the rate generated by a 30% burn. The catabolic demands of this response are huge. Prior to the era of continuous hyperalimentation at the Institute of Surgical Research, patients with greater than 40% total body surface burns lost approximately 25% of their preburn weight in the first 6 weeks post injury. Recent work by Wilmore, et al³ has shown that the ability of a thermally injured patient to maintain hypermetabolism in the face of a cold stress can be exhausted and that this exhaustion is correlated with mortality.

The stimulus, mediators, and mechanisms of end organ response that produce the hypermetabolic reaction to thermal injury are not fully understood. Wilmore, et al³ demonstrated a correlation between metabolic rate and urinary catecholamine excretion. They were further able to decrease the hypermetabolism of burned patients via a combination of alpha and beta adrenergic blockade or beta adrenergic blockade alone indicating that catecholamine is one of the more significant efferent mediators of this response. Glucagon, which is also increased in the post burned state, has been shown to be capable of increasing metabolic rate of normal as well as burned individuals.⁴ Thyroid hormone, mediator of hypermetabolism in other conditions, has been shown not to be a primary mediator.^{5,6} Burn victims have, in fact, in some situations been shown to have

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diminished thyroid hormone levels.⁷ The precise character of the mediating hormonal milieu has not been delineated. The afferent stimuli that induce this milieu are unknown. The character of end organ and cellular response are unknown. Further definition of these mechanisms might lead to an ability to better support and modulate the process.

Various groups have developed animal models to investigate the hypermetabolic response to thermal injury. Morgan, et al⁸ found insensible water loss to be significantly elevated in burned rats. Lieberman and Lansche⁹ measuring metabolic rate and insensible water loss of thermally injured rats found both to be elevated but "the rate of increase in oxygen consumption was less than the increase of caloric loss incident to the increased rate of insensibly lost water in these rats". From this and the evidence of one burned rat whose metabolic rate was returned to normal by placing a water impermeable covering over the burn they concluded that the hypermetabolic reaction to thermal injury was attributable to heat loss from the evaporation of water. Caldwell in a series of studies using a Benedict calorimeter came to similar conclusions.^{6,10} He showed a significant increase in metabolic rate in 20% burned rats relative to unburned controls studied at 24°C from the 2nd through the 65th post injury day. However, when these same animals were studied at 32°C, in the thermoneutral range for rats, there was no difference in the metabolic rate of the 2 groups. His interpretation, like that of Lieberman and Lansche, was that post burn hypermetabolism in rats is providing energy, needed to offset evaporative heat losses. Human studies by Wilmore, et al^{1,2} and by Barr, et al¹¹ clearly demonstrate that the human hypermetabolic response post thermal injury is not abolished by raising ambient temperature to thermoneutral, 32°C. Further, Zawacki, et al¹² showed that covering the burn wound with a water impermeable membrane did not decrease caloric utilization. In man evaporative water loss is not the primary stimulus of the hypermetabolic response but is elevated in parallel with it and may be the method by which the increased heat generated in response to other signals

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is dissipated. In recent animal studies^{13,14} Farkas, et al showed small increases in oxygen consumption in 59 rats with 27% burns over those of paired controls. This increase was significant in animals studied at 32°C in an atmosphere with a humidity of greater than 60%. Oxygen consumption was less in both burned and control animals at 32°C than at 24°C.¹³ Aikawa, et al studying the hypermetabolic response of guinea pigs with 11% total body surface burn at 24.8°C reported increases in post burn metabolic rates which were correlated with increases in urinary catecholamine excretion. In this study elevation in total energy production could not be explained by commensurate elevations in measured evaporative heat loss. They concluded that increases in evaporative heat loss post thermal injury were secondary and "could at most account for a small portion of the post burn hypermetabolism".¹⁴

The objective of this investigation was to develop a reliable and easily reproducible animal model of the post burn hypermetabolic response of humans that would allow investigations of the stimulus, mediators, and mechanisms of end organ response to that reaction.

MATERIALS AND METHODS

Various groups of animals were burned over 20, 40, and 60% of their total body surface by immersion in boiling water as previously reported by Walker and Mason.¹⁵ The animals were anesthetized with intraperitoneal pentobarbital, (with the exception of guinea pigs which were anesthetized with inhalation penthrane). The animals were clipped of fur prior to burning. Weight-paired control animals were also anesthetized and clipped of fur, they were then dipped in tepid water. All animals, except as noted below, were resuscitated with 1 cc of Ringer's lactate/kg/% burn injected intraperitoneally at the time of burning and with another equal injection 8 hours post burn. Whole body oxygen consumption was determined over 1 to 2 hour periods prior to burn (or sham manipulation) and at intervals of 1 to 4 days thereafter to approximately 45 days post burn. Control animals were re-clipped of fur twice each week. Oxygen consumption was studied by placing animals in air-tight desiccator jars over a layer of soda-lime, (a CO₂ absorbent). The jar was connected in series to an Erlenmeyer flask equilibrated with 100% oxygen. The flask was connected in series to a water bath in which all apparatus was submerged. The bath temperature and the ambient temperature in the metabolic chambers were kept constant at the predetermined level $\pm 1.5^{\circ}\text{C}$. As the animals breathe, CO₂ is trapped by the soda-lime, and a vacuum is created which, after overcoming the surface tension of the system, pulls oxygen from the flask to the jar and water from the bath to

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14. Aikawa N, Caulfield JB, Thomas RJS, Burke JF: Postburn hypermetabolism: Relation to evaporative heat loss and catecholamine level. *Surg Forum* 26: 74, 1975.

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the flask in a volume proportional to the amount of oxygen consumed. Sixteen jars are run simultaneously, 8 experimental animals and 8 weight-paired controls. The length of the experiment in minutes, the volume of water pulled into the Erlenmeyer flask in ccs, the weight of the animal in grams, the ambient temperature in degrees centigrade in the metabolic chambers and the barometric pressure at the time of the experiment were recorded. From these measurements, oxygen consumption/unit weight/unit time could be calculated after correcting for standard temperature and pressure. All animals were studied on different days at ambient jar temperatures of 27°C and at ambient temperatures of 32°C, thermoneutral. Oxygen consumption/unit time of experimental animals were compared with oxygen consumption of weight-paired controls over periods of time post injury by the Students unpaired "t" test, and the Scheffe modification of the "t" test when required.

RESULTS

The Effect of a 60% Thermal Injury on the Growth Rate of Rats Burned at 180 gms of Weight: The growth rate of 50 rats given a 60% burn when they weighed 180 gms was compared with that of 50 sham burned rats weighing 180 gms. As seen in Figure 1, the regression line defining post burn day versus weight for burned animals from the date of burn to the 80th postburn day is higher than and has a steeper slope than the regression line defining the control animals. The line defining the rate of growth of the burned animals from the 7th to 80th post burn days (Figure 2) is, however, seen to be parallel to but significantly elevated over that of the controls. This indicates that when rats are burned at 180 gms, a point early on their linear growth phase, they undergo a growth arrest of approximately 7 days after which they regain at a normal rate. At any given time post burn, however, the burned rats are lighter than controls weight-paired at the time of burning. Therefore, any comparison affected by weight of the animals must be corrected for this discrepancy.

Comparison of the Metabolic Rate of 60% Thermally Injured Growing Rats with that of Predicted Weight-Paired Controls from the 1st to 60th Post Burn Day with all Animals being Studied at an Ambient Temperature of 27°C. The amount of oxygen consumed/hour is plotted against weight for 40 control rats sham burned at 180 gm weight and studied every 3 days post sham at an ambient temperature of 27°C (Figure 3). The regression line defined by the equation $Y = 160.70 + 0.7709X$ is a fair predictor of this relationship, (r^2 value = 0.67). If one divides the observed oxygen consumption/hour of control rats of a given weight by the oxygen consumption/hour that would be predicted for given weights by the above regression line and compares these ratios to post burn day, a relationship with slope confidence limits of -0.0019 to 0.0005 and a Y intercept of 1 is obtained (Figure 4). This confirms the validity of the above regression line as a predictor of normal oxygen consumption/unit weight. In Figure 5, the oxygen consumed/hour by a group of 40, 60% burned rats studied at an ambient temperature of 27°C, divided by the oxygen consumption that would be predicted for a normal rat of the same weight, under the same conditions, is plotted against post burn day. A curve is defined that is very similar to that obtained for thermally injured patients. Metabolic rate increases from a preburn level which is equivalent to the controls to an

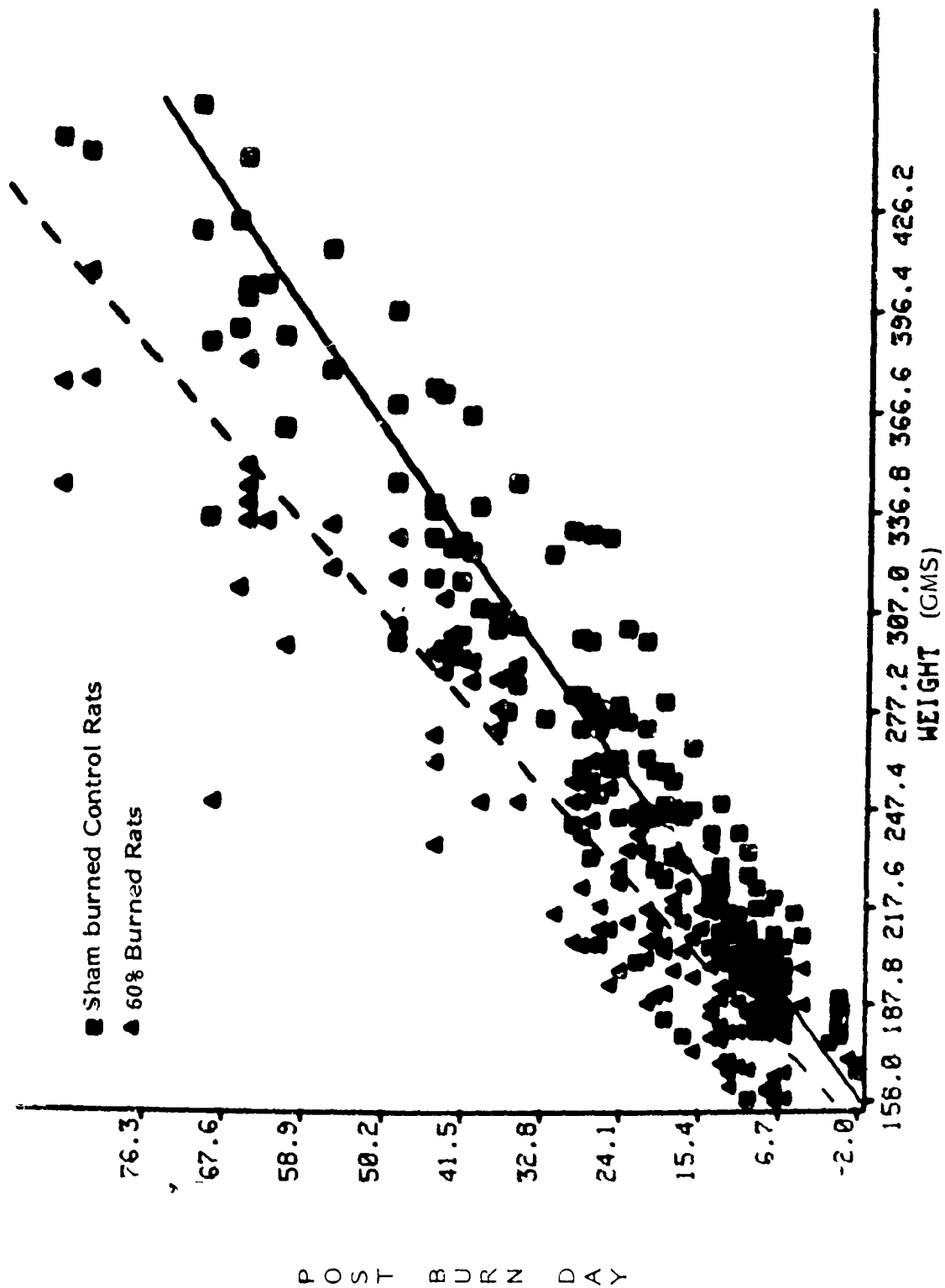


FIGURE 1

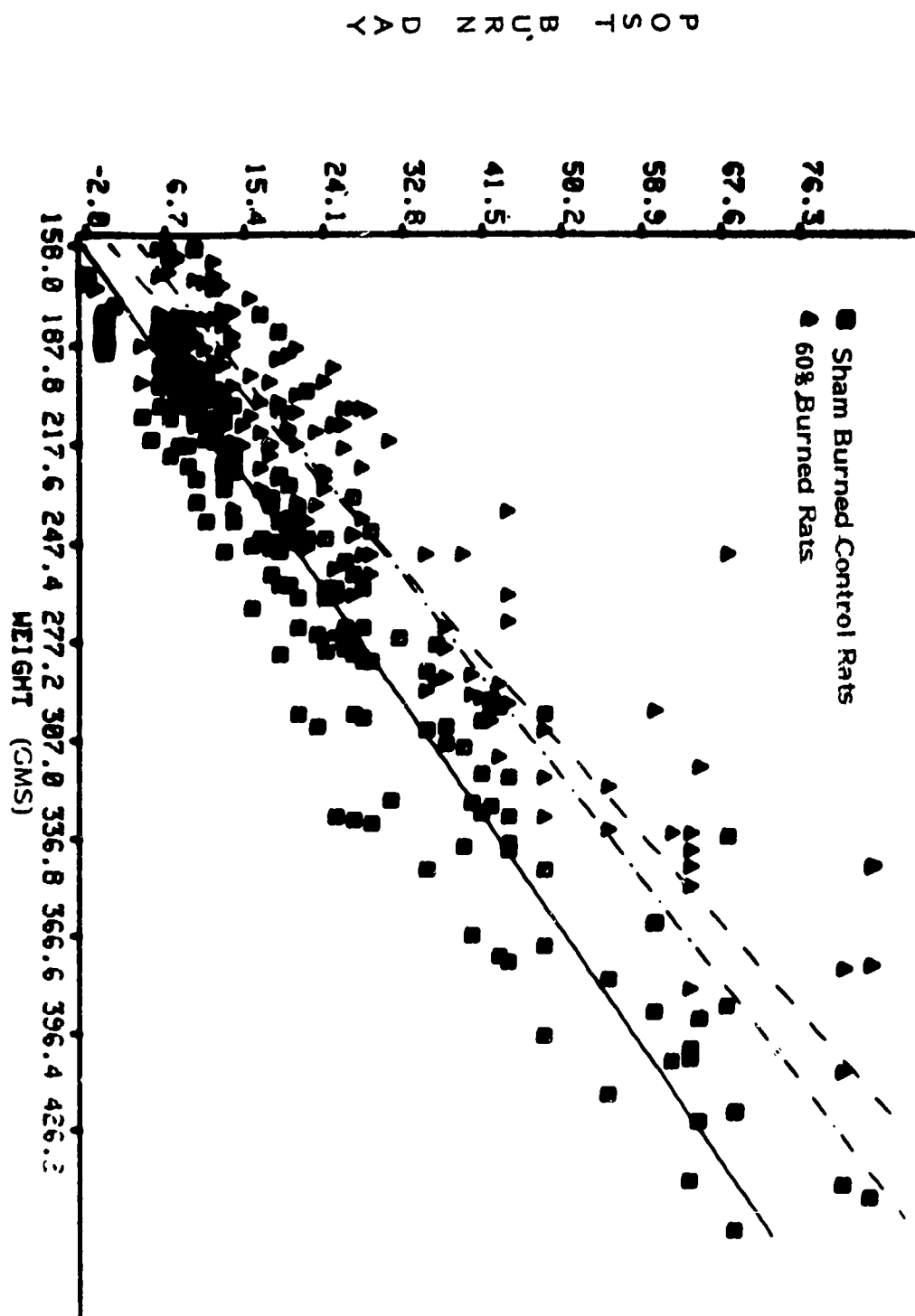
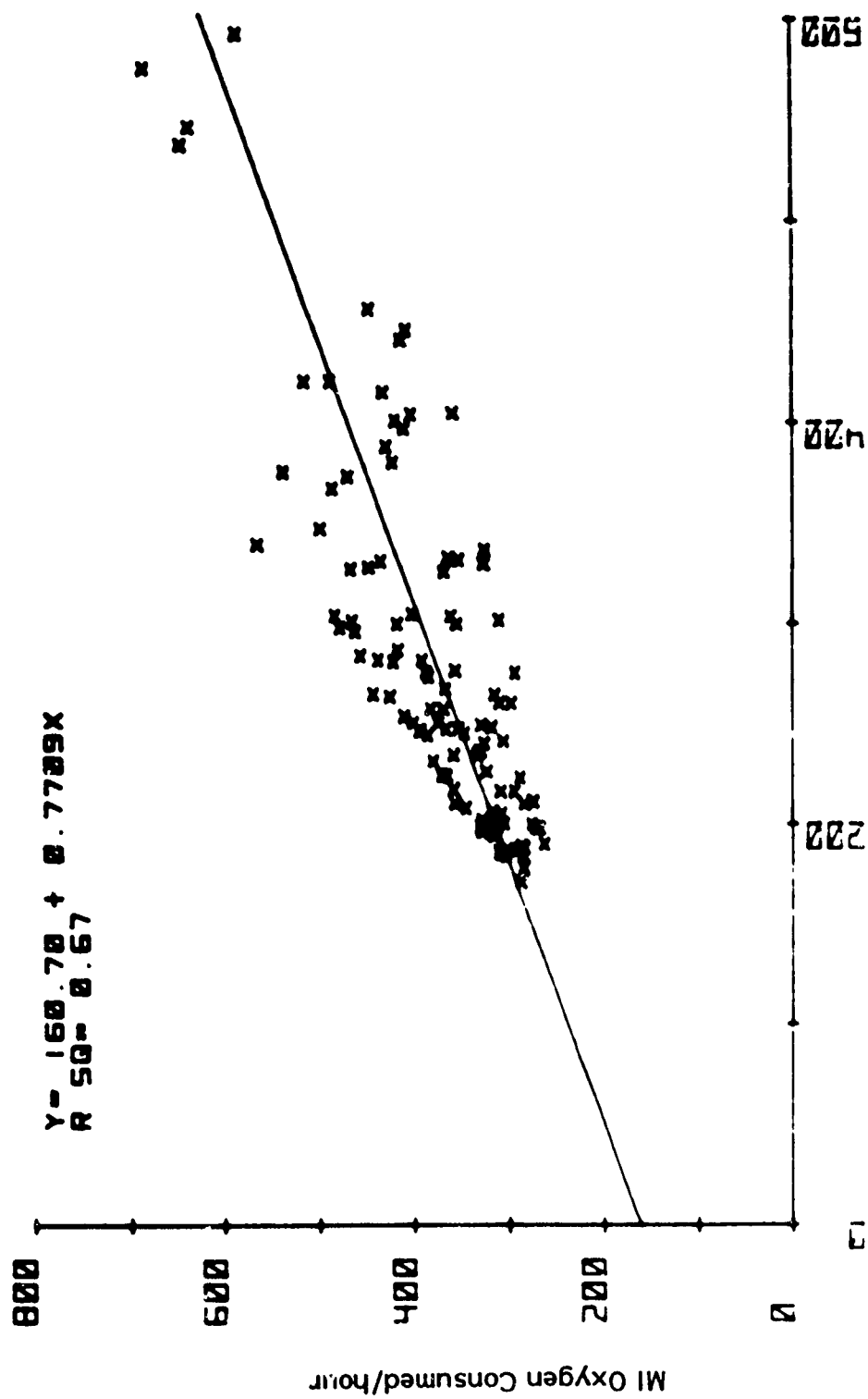
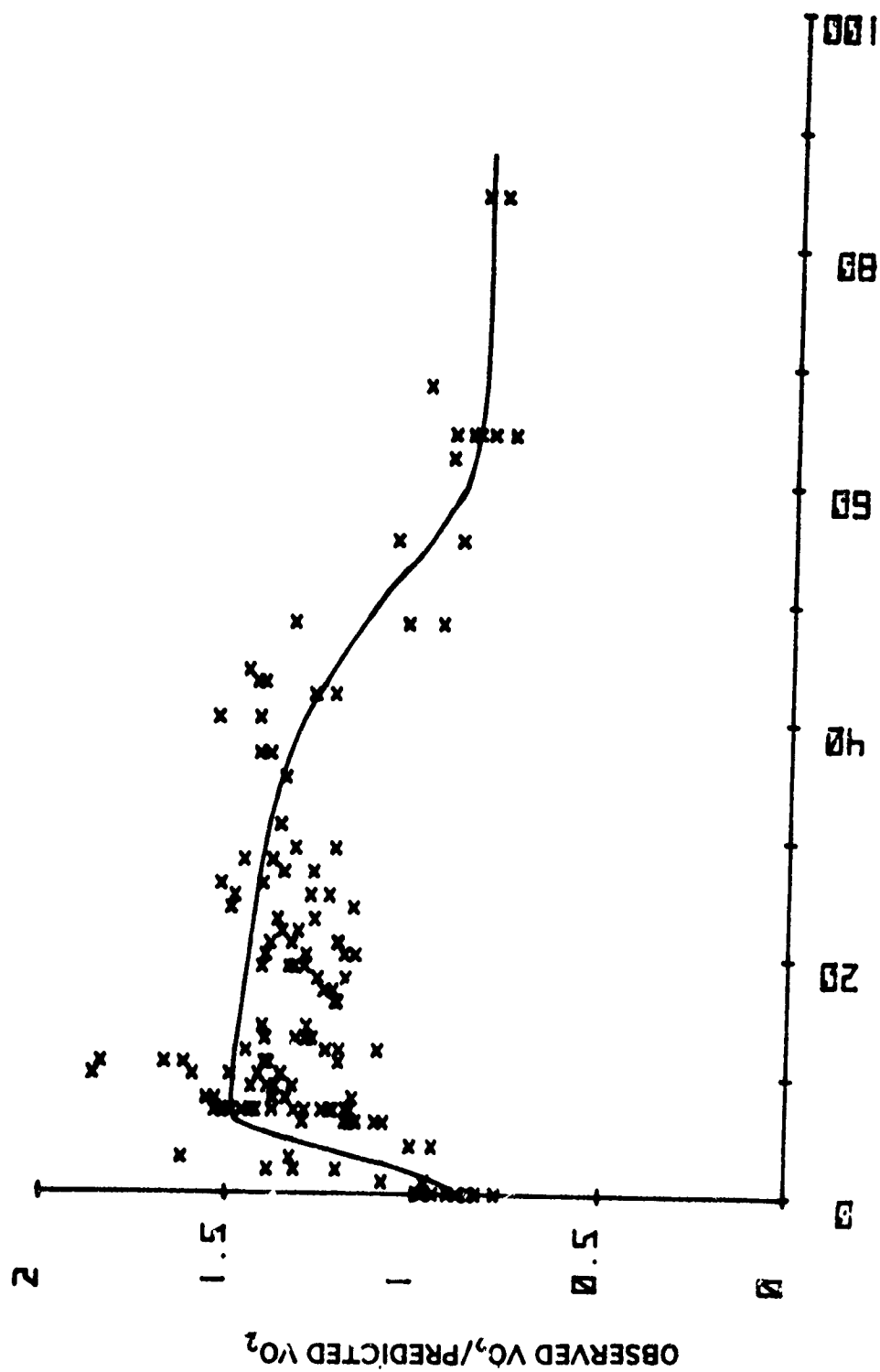


FIGURE 2





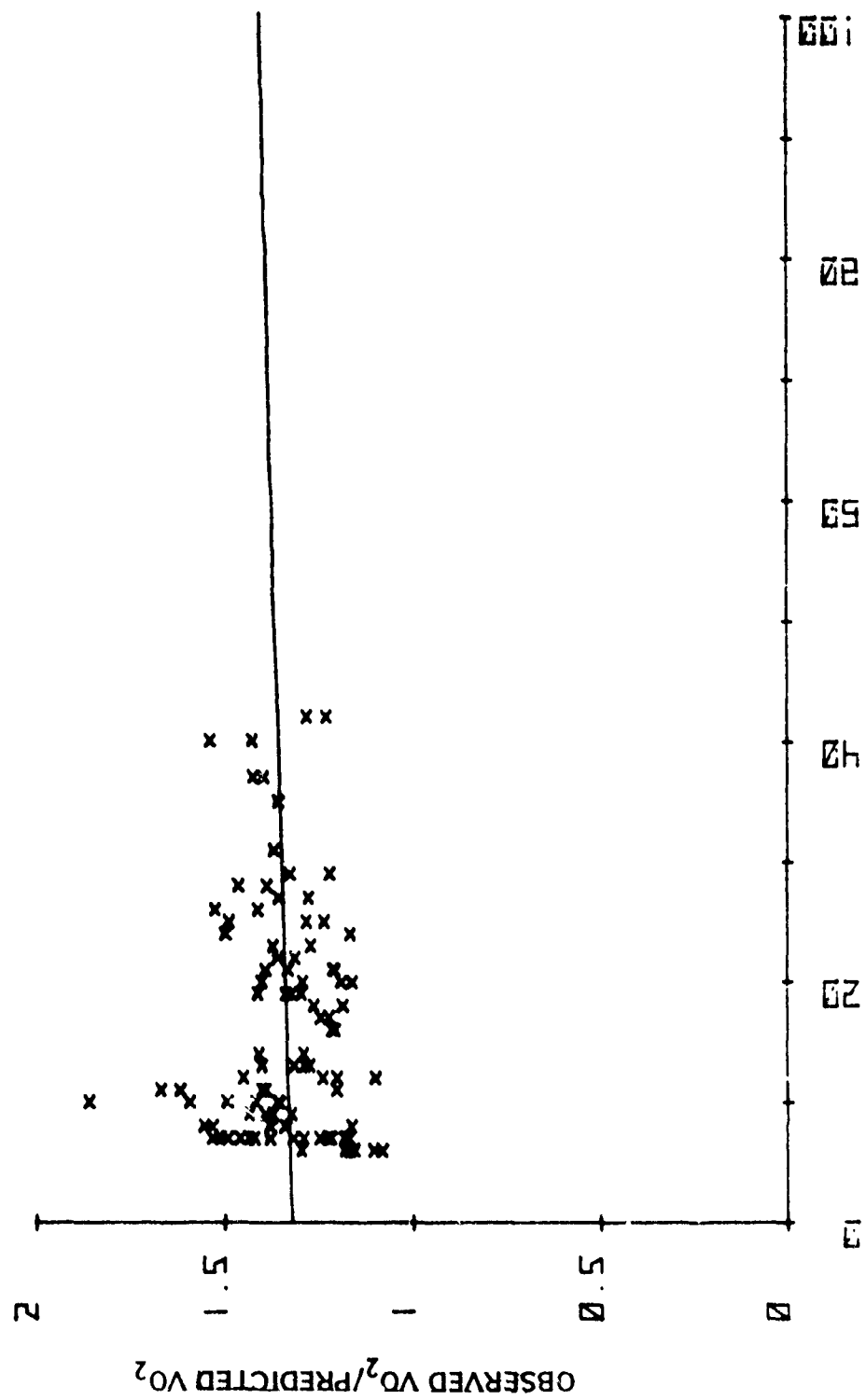
60% Burned Rats, 27° C

FIGURE 5

elevated plateau which is relatively constant from the 5th to the 45th post burn day, at which time the metabolic rate gradually decreases to control levels by the 60th post burn day. If the ratios of observed burn over predicted normal oxygen consumption are plotted against post burn day from the 5th to the 45th post burn day (Figure 6), they are found to define a horizontal line with a Y intercept of 1.36. If these values are compared with the observed/predicted ratios of the controls, this post burn hypermetabolic response is found to be highly significant ("t" value 18.7 with 202 degrees of freedom $p < 0.0001$ - Figure 7). At 27°C growing rats have an increase in metabolic rate from the 5th through the 45th post burn day of approximately 34% above controls of the same weight.

Comparison of the Metabolic Rate of 60% Thermally Injured Growing Rats with that of Predicted Weight-Paired Controls Studied at an Ambient Temperature of 32°C. The amount of oxygen consumed/hour is plotted against weight for 40 control rats sham burned at 180 gm weights and studied every 3 days post sham at a 32°C ambient temperature, thermoneutral, (Figure 8). A regression line is defined by the equation $Y = 74.6441 + 0.0072X$ with an r^2 value of 0.86. Dividing the observed oxygen consumption/hour of control rats of given weights by the amount of oxygen that would be predicted from the above equation to be consumed by a rat of that weight, one obtains a line with slope confidence limits of -0.00117 and + 0.0011 with a Y intercept of 1 (Figure 9). The ratio of oxygen consumed/hour by a group of 40, 60% thermally injured rats studied at the ambient temperature 32°C, divided by the predicted amount of oxygen that would be consumed by control rats of similar weight under similar conditions, again defines a curve very similar to that of thermally injured humans (Figure 10). The metabolic rates rise over the first 5 days post burn to an elevated plateau 32% higher than that of controls. The elevation in metabolic rate is relatively constant from the 5th to the 45th post burn day after which it gradually decreases to normal by the 60th post burn day. Comparison of the metabolic rates of the burned rats studied at 32°C with normals under similar conditions (Figure 11), shows the 32% increase in the burned animals' metabolic rate over controls to be highly statistically significant (unpaired "t" value 15.5 with 189 degrees of freedom $p < 0.0001$). This indicates that growing rats, like humans, are hypermetabolic post thermal injury when studied at an ambient temperature of 32°C. Increasing temperature does decrease the amount of oxygen consumed by both normal and burned animals of any given weight as can be seen by the comparison of oxygen consumption/hour against weight for control animals at 32°C and at 27°C (Figure 12). The regression line defining the animals studied at 32°C is significantly lower than that defining those studied at 27°C ($p < 0.01$). The comparison of the burned rats studied at the 2 temperatures (Figure 13) also shows that the oxygen consumption is lower at the warmer temperatures; but the decrease in metabolic rate of burned animals at the higher ambient temperature is comparable to the reduction observed in the control rats.

The Effect of Animal Age on Metabolic Rate. Mature rats, that had reached the plateau of their growth phase (540 gm), were submitted to 60% thermal injuries and their metabolic rates were found to be significantly elevated over weight-paired controls from the 5th through the 26th post burn day, $p < 0.001$ (Figure 14). The increase in metabolic rate of these mature, 60% burned rats was



POST BURN DAY
60% Burned Rats, 27° C

FIGURE 6

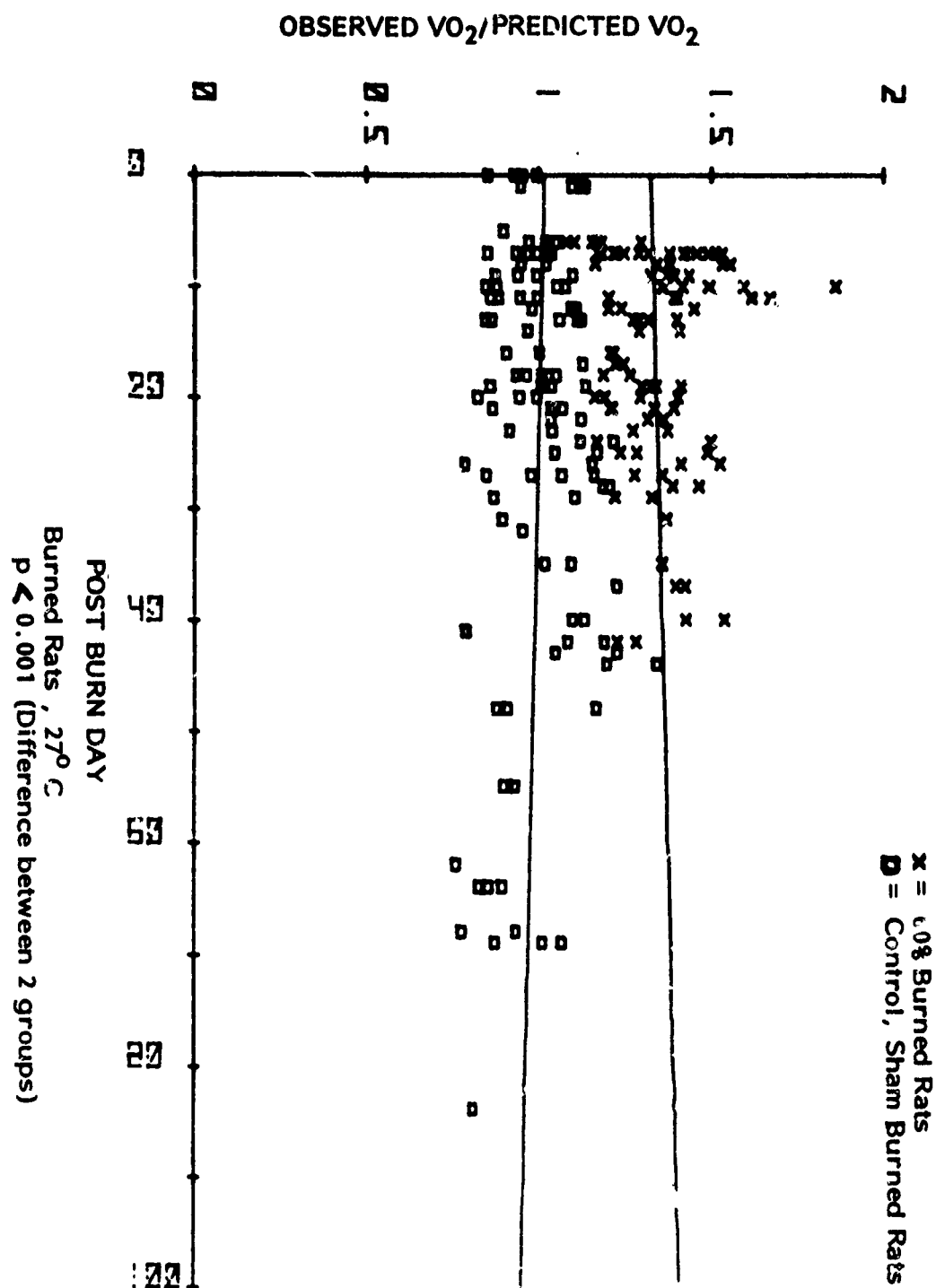


FIGURE 7

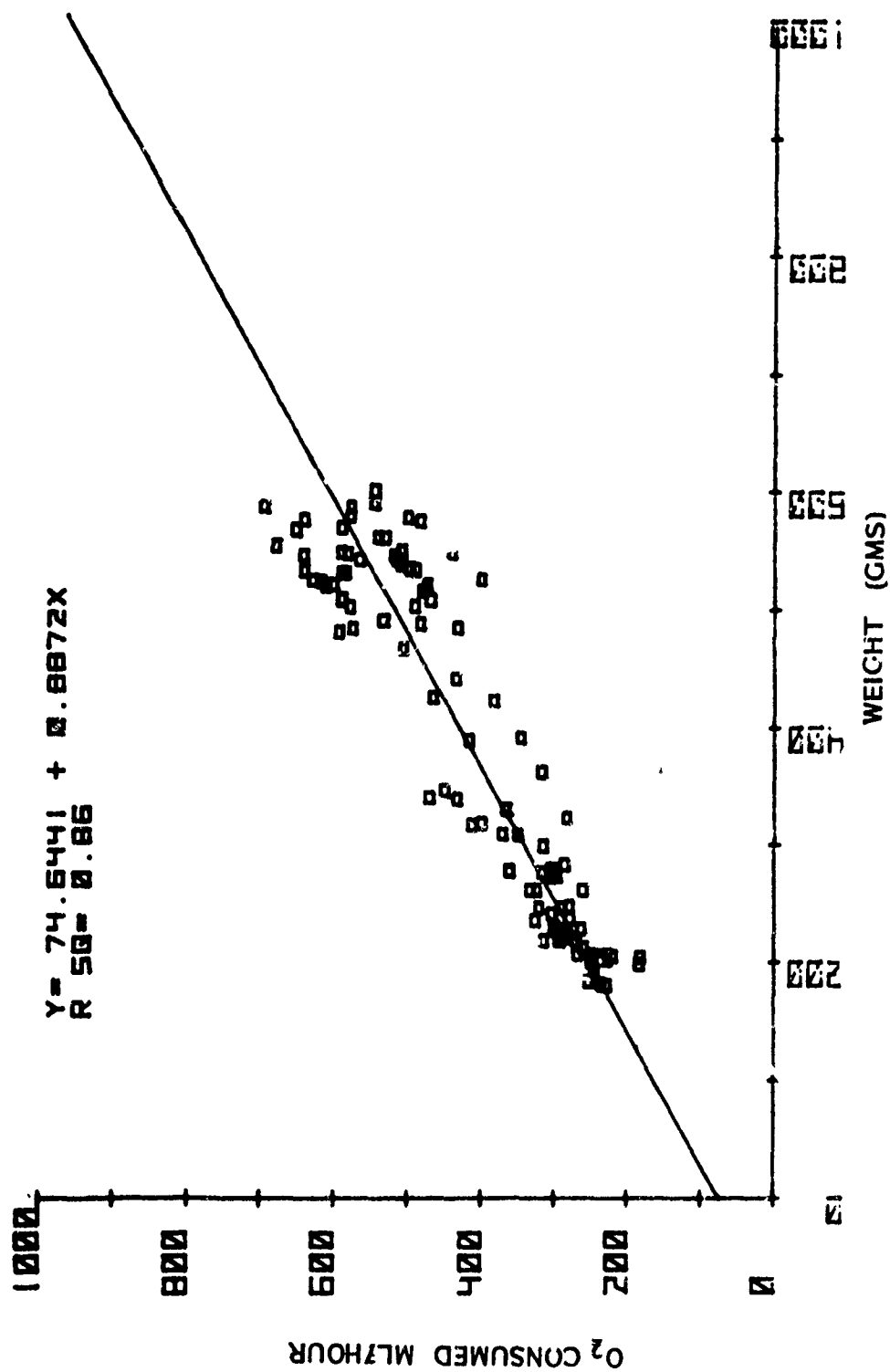
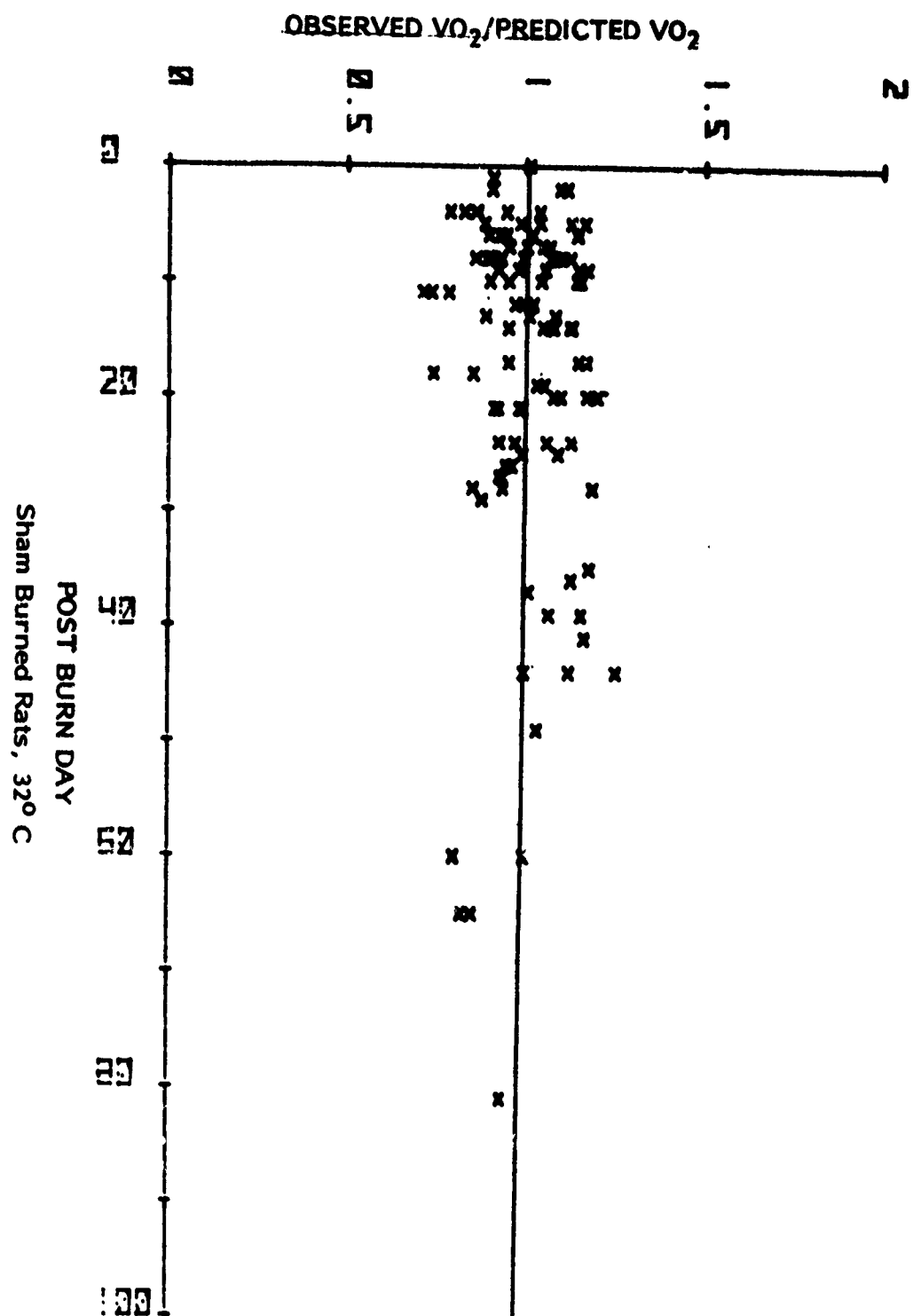


FIGURE 8



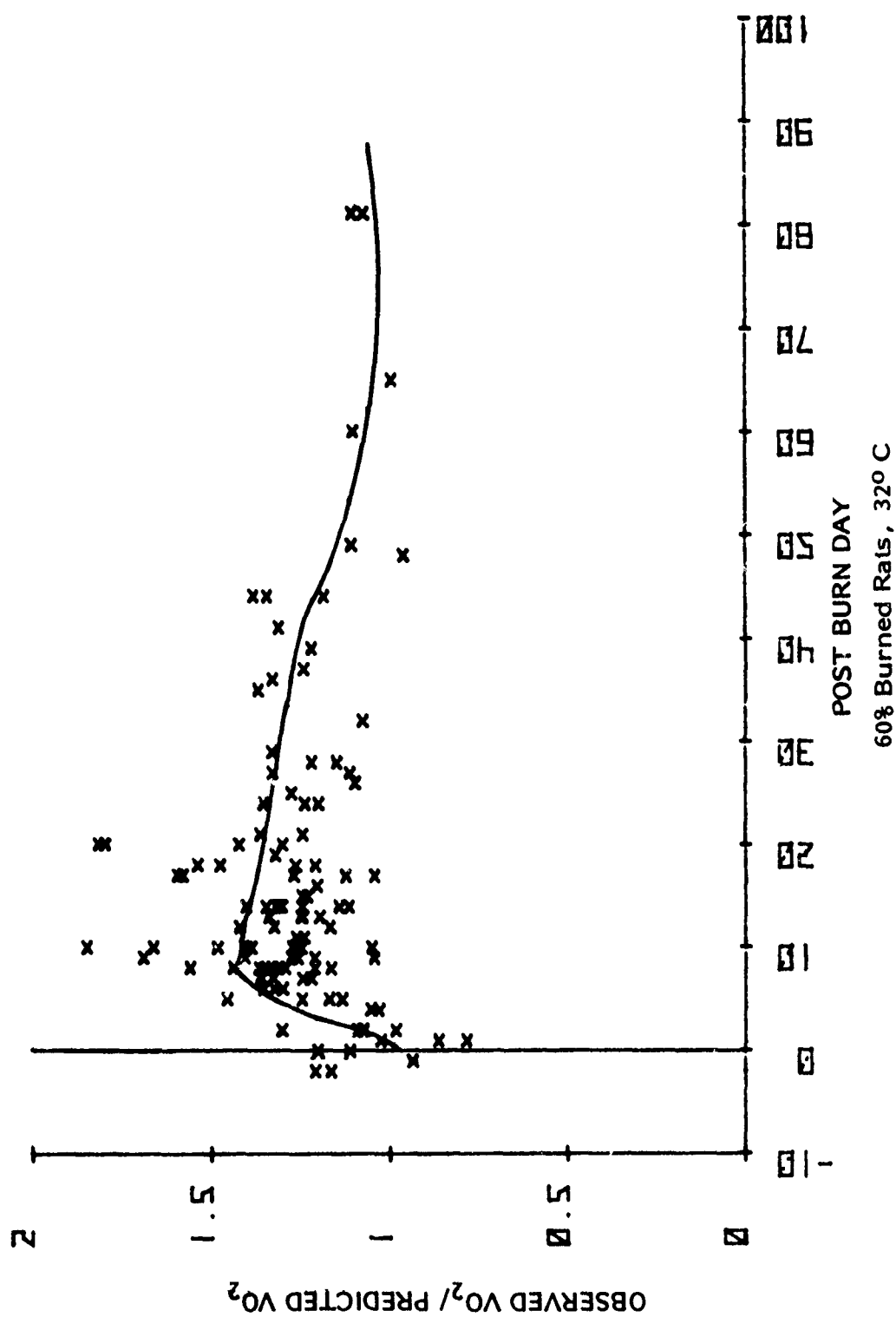
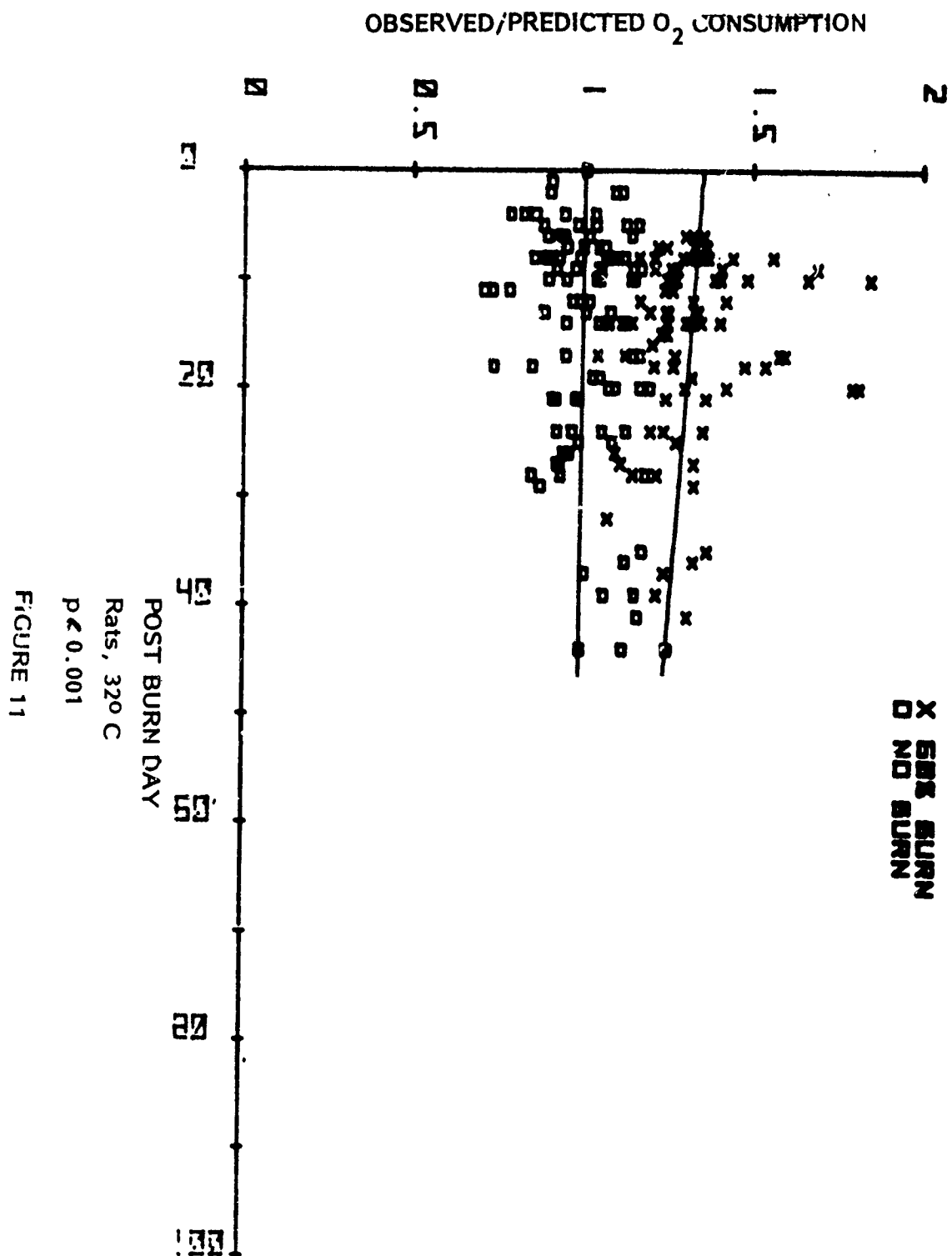


FIGURE 10



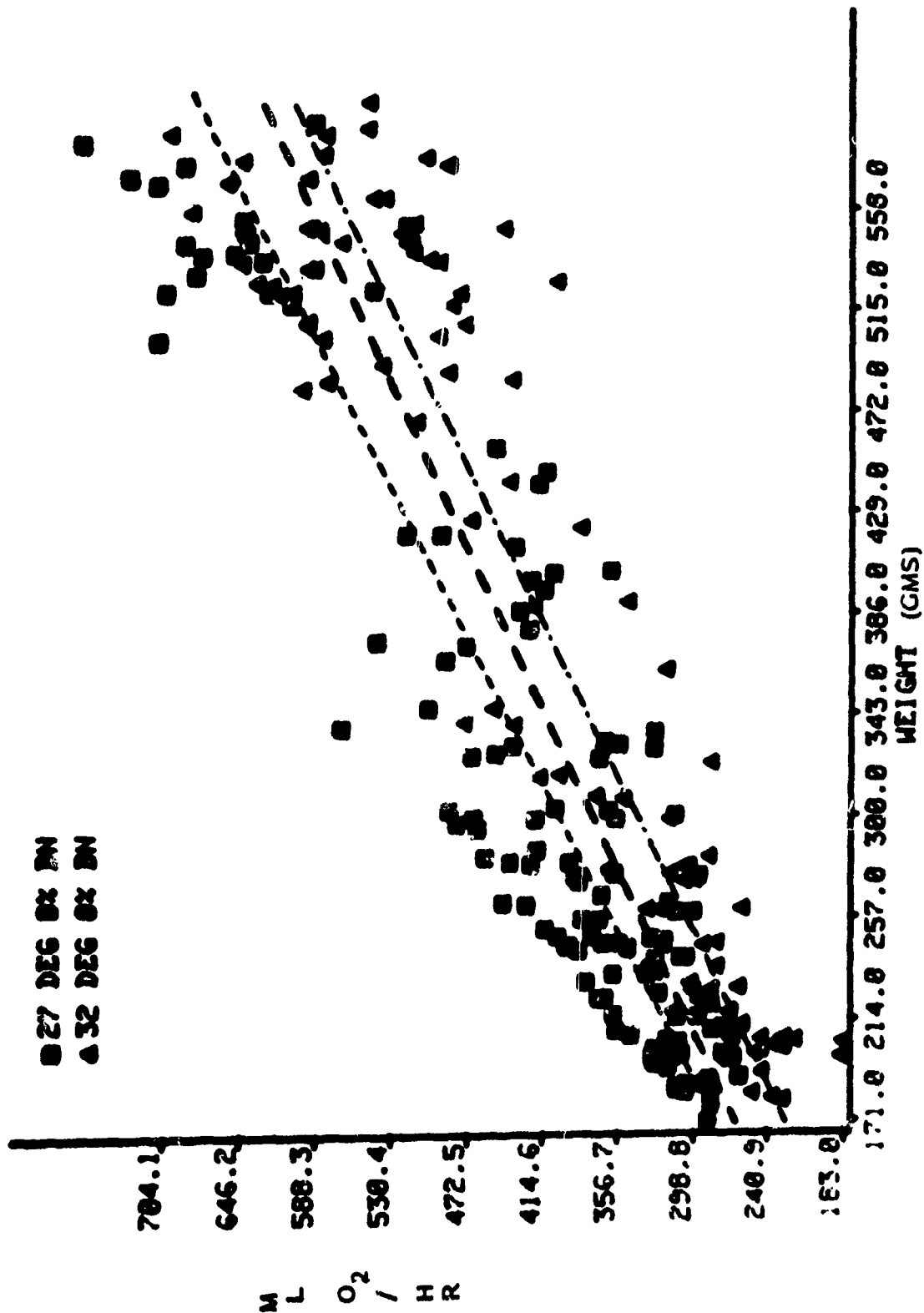
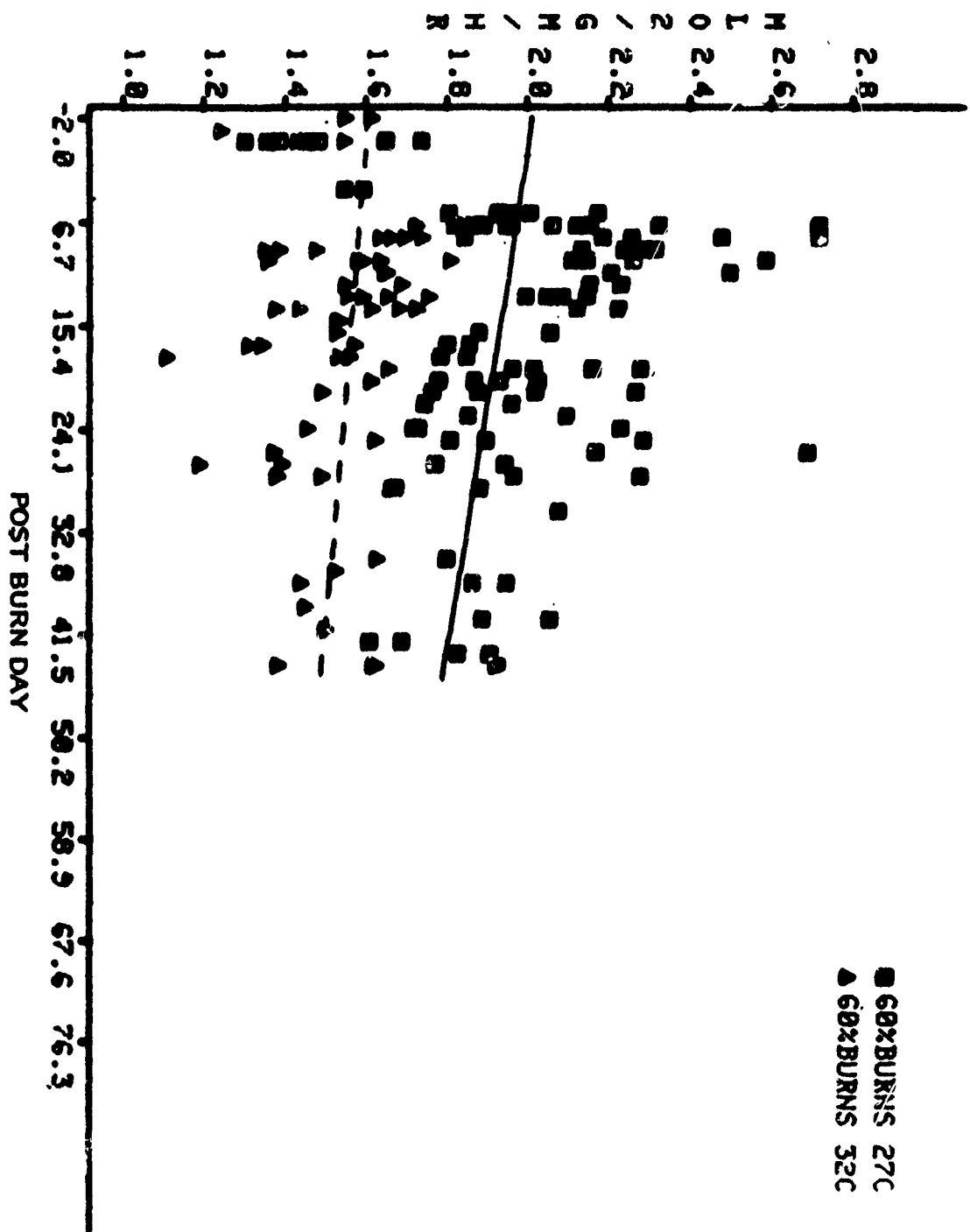


FIGURE 12



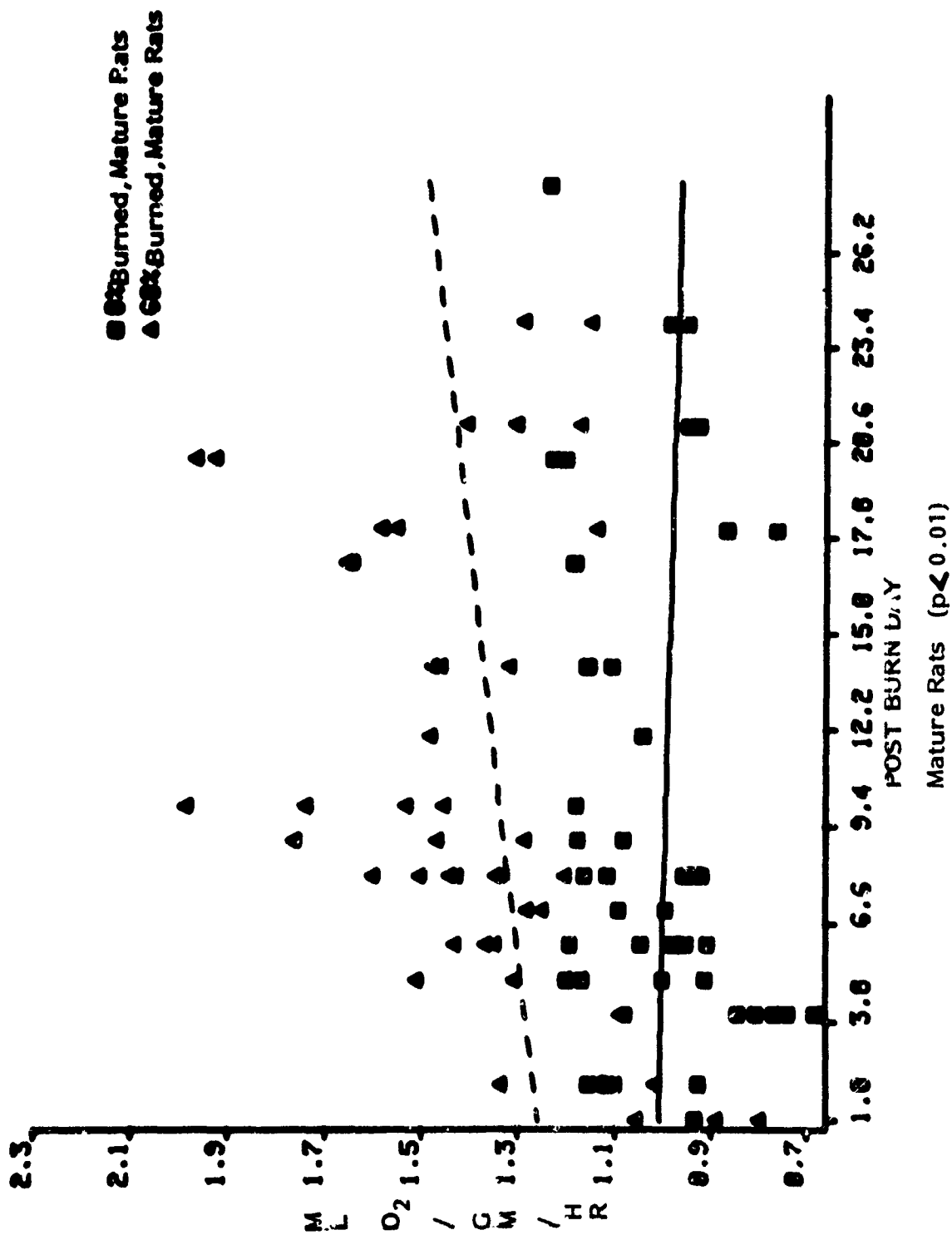


FIGURE 14

1.41 times that of their weight-paired controls. The mortality of these mature animals following a 60% thermal injury was markedly greater than that of growing rats after a similar injury. 85% of the large rats succumbed within the first 28 post burn days. Only 10 to 15% of the growing rats succumbed to a similar injury. The cause of death in the adult rats, confirmed by autopsy appearance of gram negative wound invasion and miliary hematogenous bacterial deposits, as well as several pre-death blood cultures, was gram negative wound invasion and sepsis. The frequency of death in these mature rats after a 60% burn more closely approximates the human response to thermal injury than does that of the growing rat. It is presumed that there is something protective in the anabolic milieu of the growing rat to thermal injury. The adult rat may be a better model of the human response.

Comparison of the Metabolic Rates of 40% and 60% Thermally Injured Guinea Pigs with the Predicted Metabolic Rates of Weight-Paired Controls Studied at 32°C Ambient Temperature, Thermoneutral. A group of 20 guinea pigs was given a 40% thermal injury at 280 gm body weight (an early point on the linear growth phase). A second group of 70 guinea pigs was given a 60% burn at 540 gm body weight (a point midway on the linear growth phase for guinea pigs). Both groups were compared with weight-paired controls and with each other. Figure 15 shows that the oxygen consumed/hour/unit weight of control guinea pigs studied in an ambient temperature of 32°C can be described by the equation $Y = 145.7246 + 0.5817X$ with an r^2 value of 0.66. When observed oxygen consumption/hour for animals of given weight is divided by the oxygen consumption/hour that would be predicted for that weight from the above equation and the results plotted against post sham days, a horizontal line with a Y intercept of 1 is defined as would be predicted (Figure 16). When the observed oxygen consumption of 40% burned guinea pigs is divided by that predicted for that weight and plotted against post burn day, a curve similar to that of rats and humans is defined, increasing over the first 5 days post burn to a plateau that is constant from the 5th to the 45th post burn day, gradually returning to normal by the 60th post burn day (Figure 17). If the rates of oxygen consumption for the burned animals from the 5th to the 45th post burn days are compared with the predicted normals of the animals of the same weight, the observed increase is highly significant with a $p < 0.0001$, the burns being elevated 1.31 times the controls. The 20, 60% burned guinea pigs, by the same method of analysis, were found to have an elevation of 1.39 times that of controls, again highly significant with $p < 0.0001$ (Figure 18).

The Effect of Resuscitation on Metabolic Rate Post Thermal Injury. Groups of 10 controls and 10, 60% burned growing rats were respectively resuscitated with 1 cc/kg/% burn of Ringer's lactate at the time of burn and 2 cc/kg/% burn Ringer's lactate. No differences in the degree of hypermetabolism post thermal injury could be ascertained among these four groups.

The Effect of Thyroidectomy on Metabolic Rate. Ten thyroidectomized control rats, 10 thyroidectomized 60% burned rats, 10 non-thyroidectomized control rats, and 10 non-thyroidectomized 60% burned rats were compared. All rats were 540 gm at the time of burn or sham. Metabolic rates were compared from the 5th to the 25th post burn days at which times the metabolic rates of all

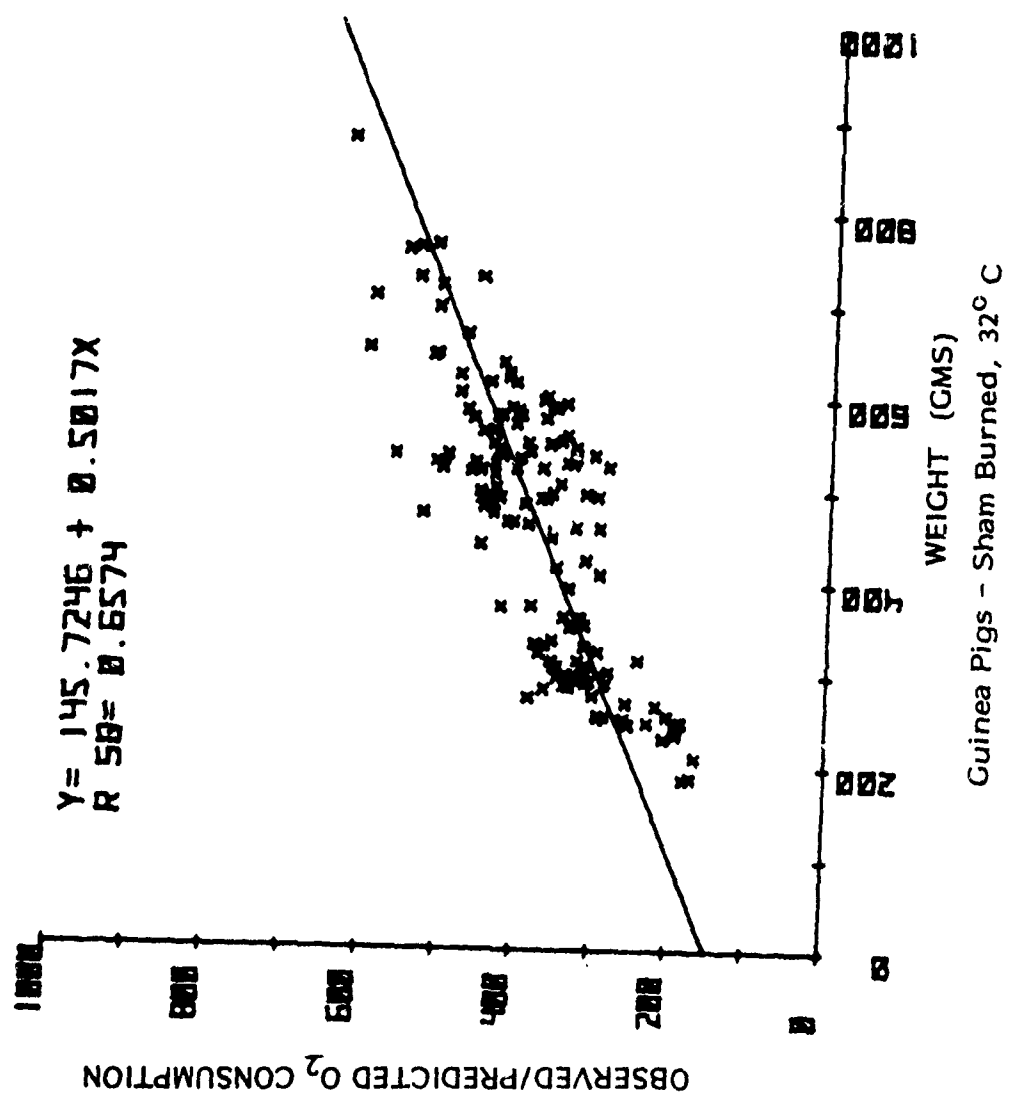
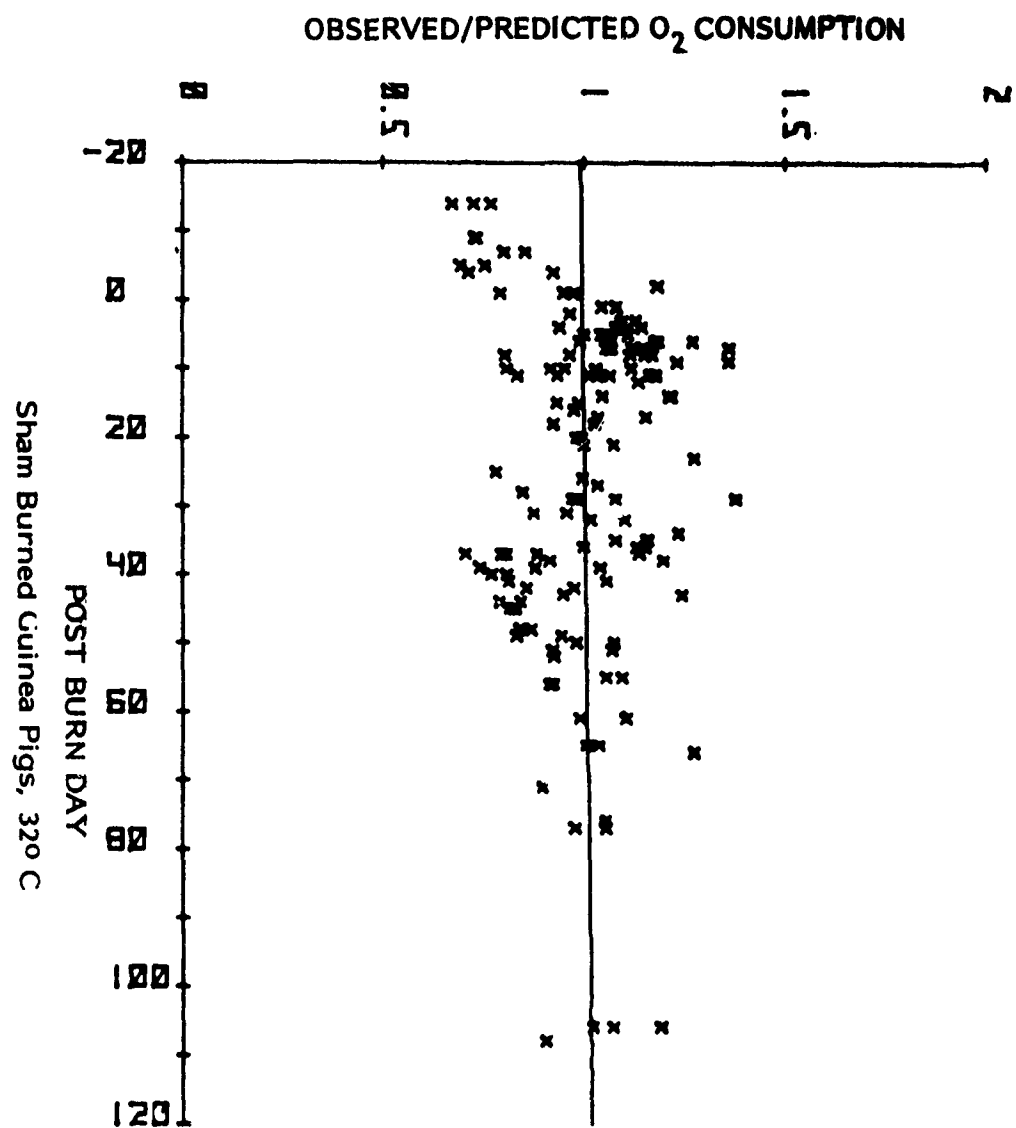
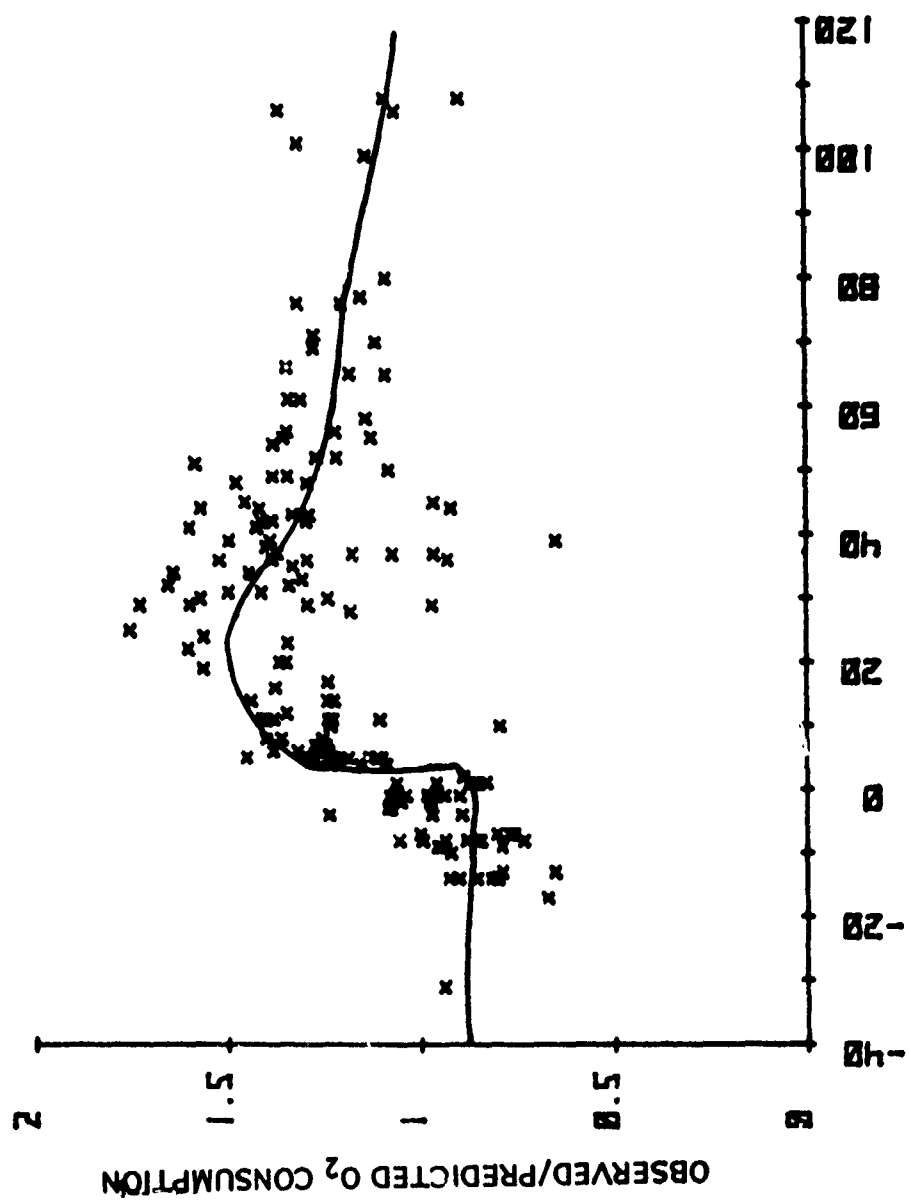


FIGURE 15





40% Burned Guinea Pigs, 32°C

FIGURE 17

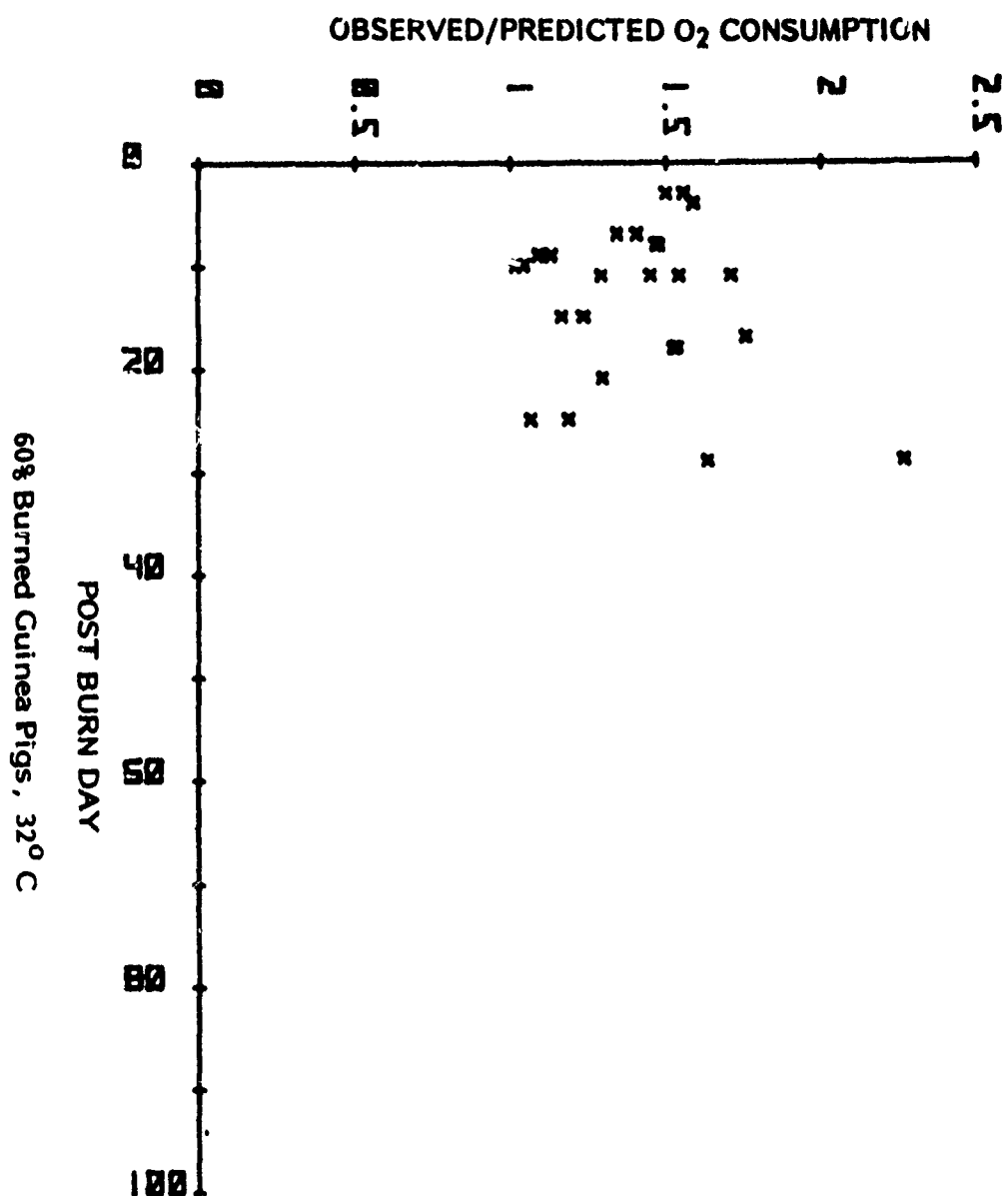


FIGURE 18

groups were found to be constant. Their ratios were: thyroidectomized unburned rats, 0.61, < thyroidectomized burned rats 1.01 = to non-thyroidectomized controls 1.00, < non-thyroidectomized burns 1.41. These results were significant with a p value of < 0.05 by the Scheffe test. This indicates that thyroid hormone levels are not a primary mediator of the post burn hypermetabolic response but do condition it.

DISCUSSION

Previous small animal models that have been developed to simulate the human post burn hypermetabolic response have shown significant increases in oxygen consumptions with various burn sizes, when the animals were studied at relatively cold environments (24°C).^{8,9,10,14} Only one study has shown consistent increases in metabolic rate when the animals were studied at thermoneutral 32°C,³ and this increase was quite small. The human post burn hypermetabolic response is clearly not temperature dependent.^{1,2} The increases in metabolic rate in humans can not be explained by increased energy requirements generated by evaporative heat loss,^{1,2,12} as has been the explanation for the increased metabolic rates in many of the above animal studies.^{8,9,10} The present study establishes an animal model using rats and guinea pigs of varying stages of maturity, all of which showed large (on the order of 130-140% of controls) and significant ($p < 0.0001$ for all groups) post burn hypermetabolic reactions at thermoneutral (32°C). The metabolic responses of these animals was temperature sensitive as both normal and burned animals showed lower metabolic rates at 32°C than at relatively cold 27°C. But the decreases in metabolic rate were proportional for the burned and unburned animals. The characteristics of the metabolic rate versus post burn day of the animals studied in this project closely simulate those previously reported for humans,^{1,2} increasing to a plateau elevation from the 5th to the 45th post burn day and then gradually returning to normal as the burn wound heals.

The reason this study shows increases in metabolic rates of burned animals is probably burn size related.¹³ The largest injury inflicted in earlier studies was the 27% burn of Farkas,¹³ our burns were 40 and 60%. All previously studied animals were on their linear growth phase, burns being applied to rats when their weight was 180 to 220 gm. A 20-30% burn is fatal to only 5-15% of animals in this anabolic milieu and probably does not represent a sufficient stress to institute a hypermetabolic response.

PRESENTATIONS and/or PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY					1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
					DA OE 6977	76 10 01	DD-DR&E(AR,636)	
3 DATE PREV SUMRY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8A DES'N INSTR'N	8B SPECIFIC DATA- CONTRACTOR ACCESS		9 LEVEL OF SUM
75 07 01	H. TERM	U	U	NA	NL	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>		A. WORK UNIT
10 NO /CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61102A	3A161102B71P		02	066			
b. CONTRIBUTING								
c. CONTRIBUTING								
11 TITLE (Precede with Security Classification Code) ^a								
(U) Evaluation of Calcium Metabolism in Burned Troops (44)								
12 SCIENTIFIC AND TECHNOLOGICAL AREAS ^a								
003500 Clinical Medicine								
13 START DATE		14A ESTIMATED COMPLETION DATE		15 FUNDING AGENCY		16 PERFORMANCE METHOD		
74 01		76 09		DA		C. In-House		
17 CONTRACT/GRANT				18 RESOURCES ESTIMATE		19 PROFESSIONAL MAN YRS		20 FUNDS (in thousands)
Not Applicable				PREEXISTING		.3		1.0
a. DATES/EFFECTIVE:				76				
EXPIRATION:				7T		.1		2
b. NUMBER ^a				FISCAL				
c. TYPE				YEAR				
d. KIND OF AWARD				CURRENT				
e. AMOUNT:								
f. CUM. AMT.								
18 RESPONSIBLE DOD ORGANIZATION				19 PERFORMING ORGANIZATION				
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research				
ADDRESS ^a Fort Sam Houston, Texas 78234				ADDRESS ^a Fort Sam Houston, Texas 78234				
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)				
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21 GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER				
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS				
				NAME: William D. Myers, LTC, MC				
				NAME: Harry R. Jacobson, MAJ, MC				
				DA				
22 KEYWORDS (Precede EACH with Security Classification Code)								
(U) Calcium metabolism; (U) Burns; (U) Renal failure; (U) Hypocalcemia; (U) Humans								
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROBLEM (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)								
23. (U) The objective of this study is to determine the mechanisms of hypocalcemia and hypocalciuria in thermally injured patients. In addition the role of calcium in the production of renal impairment in thermally injured troops is to be assessed.								
24. (U) Calcium balance studies will be performed on burn patients, with all calcium intake and output measured. If, as we expect, the patient is in positive calcium balance, we will investigate the skin as the site of calcium deposition. Skin biopsies and eschar biopsies will be performed and analyzed for calcium. In addition, parathyroid hormone levels will be determined, and the response to the development of hypocalcemia will be documented. This will be correlated with any functional change in renal metabolism.								
25. (U) 75 07 - 76 09 This protocol has been completed with the entry of 20 patients into the study. To date the results of the serum and urine studies have been tabulated but the results from the dietitian concerning the intake of calcium and phosphorus are not yet available. In addition, the studies of parathormone to be performed by Dr. Goldsmith at the Audie Murphy Veteran Administration Hospital in San Antonio have not yet been completed due to the temporary interruption in his assay procedure. It is expected that the results will be tabulated and conclusions drawn within the next several months.								

Available to contractors upon originator's approval

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65
AND 1498 1 MAR 55 (FOR ARMY USE) ARE OBSOLETE

TERMINATION

**PROJECT NO. 3A161102B71P-02 BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY**

REPORT TITLE: EVALUATION OF CALCIUM METABOLISM IN BURNED TROOPS

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators

**Richard H. Merrill, MD, Lieutenant Colonel, MC
William D. Myers, Lieutenant Colonel, MC
Harry R. Jacobson, Major, MC**

Reports Control Symbol MEDDH-288(RI)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: EVALUATION OF CALCIUM METABOLISM IN BURNED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Richard H. Merrill, M.D., Lieutenant Colonel, MC
William D. Myers, M.D., Lieutenant Colonel, MC
Harry R. Jacobson, MD, Major, MC

Reports Control Symbol MEDDH-288 (R1)

This study has been completed with the entry of 20 patients into the study. To date the results of the serum and urine studies have been tabulated but the results from the dietitian concerning the intake of calcium and phosphorus are not yet available. The observed early hypocalcemia has been a consistent observation. Skin calcium levels have not been elevated so the calcium is either being excreted in the urine and/or stool, or being sequestered. In view of the observations with acute renal failure where it tends to be elevated, it is unlikely that the bone is acting as a dump. The studies of parathormone (PTH) to be performed by Dr. Goldsmith at the Audie Murphy VA Hospital, San Antonio, have not been completed due to the temporary interruption in his assay procedure. It is expected that the results will be tabulated and the conclusions drawn within the next several months. It is hoped that a method for measuring urinary CAMP will be developed at USAISR shortly, and these studies will support the PTH data.

Calcium
Burns
Renal failure
Hypocalcemia
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA CG 6952	76 10 01	DI-DR&E(AR)636	
3. DATE PREV. SUMM ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. USER INSTR ^a	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	61101A	3A161101A91C		00		082	
B. CONTRIBUTING							
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Laboratory Investigation of the Mechanisms of acquired Leukocyte Dysfunction Following Thermal Injury (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 12		Cont		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE		EXPIRATION		PRECEDING		FUND (In thousands)	
				76		1.0	
B. NUMBER ^a				FISCAL		4	
				7T		.3	
C. TYPE		D. AMOUNT		YEAR			
				COUNTRY			
E. KIND OF AWARD:		F. CUM. AMT.		77		1.0	
						20	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				Microbiology Branch			
				ADP 434: Fort Sam Houston, Texas 78234			
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				SOCIAL SECURITY ACCOUNT NUMBER			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
FOREIGN INTELLIGENCE NOT CONSIDERED				NAME: Arthur D. Mason, Jr., MD			
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Burns; (U) Leukocytes; (U) Glucose oxidation; (U) Latex phagocytosis							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
23. (U) Efforts will be made to establish one or more metabolic basis for acquired leukocyte dysfunction following thermal injury. Establishment of specific nutritional or environmental effects may allow for corrective management.							
24. (U) Initial efforts will be to measure glucose metabolism in normal and burned patients leukocytes. Purified and washed granulocyte populations will be examined for oxidation of Carbon 14 labeled glucose. Hexose monophosphate shunt and glycolysis activity will be estimated by release of ¹⁴ CO ₂ from respectively 1- ¹⁴ C glucose. Measurements will be made on resting and latex particle (≈0.8 u) stimulated cells.							
25. (U) 75 12 - 76 09 A standardized latex concentration response assay has been developed. Normal values and confidence limits have been established. Examination of patients' cells has begun with eight patients studied to date. In both stimulated and unstimulated leukocytes from burn patients both pathways of glucose metabolism were suppressed.							

PROGRESS REPORT

**PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH**

**REPORT TITLE: LABORATORY INVESTIGATION OF THE MECHANISMS OF
ACQUIRED LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL
INJURY**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Albert T. McManus, Jr, Captain, MSC
Thomas J. Lescher, MD, Major, MC
Arthur D. Mason, Jr, MD
Basil A. Pruitt, Jr, MD, Colonel, MC**

Report Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH

REPORT TITLE: LABORATORY INVESTIGATION OF THE MECHANISMS OF
ACQUIRED LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL
INJURY

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Albert T. McManus, Jr, Captain, MSC
Thomas J. Lescher, MD, Major, MC
Arthur D. Mason, Jr, MD
Basil A. Pruitt, Jr, MD, Colonel, MC

Report Control Symbol MEDDH-288 (R1)

The metabolic alterations underlying postburn anomalies of granulocyte function have been studied by examining ^{14}C -glucose oxidative metabolism in granulocytes from burned patients under serum free, chemically defined conditions. Granulocytes from burned patients and controls were prepared from heparinized (10 units/ml) venous blood by dextran sedimentation, NH_4Cl treatment, and hypaque-ficoll density gradient centrifugation. Cells were examined for $^{14}\text{CO}_2$ production from either ^{14}C -1-glucose or ^{14}C -6-glucose during a 60 minute incubation at 37°C with or without an optimal stimulatory dose of 0.79μ latex particles. Reaction mixtures contained $2-4.5 \times 10^6$ granulocytes/ml and a specific activity of $1 \mu\text{Ci}/5.5 \mu\text{M}$ glucose/ml. Phagocytosis of latex particles in the absence of serum was confirmed by scanning electron microscopic observation.

Examination of 33 normal male volunteers, ages 20-43, indicated $^{14}\text{CO}_2$ production values from C-1 or C-6 glucose to be distributed according to a log normal distribution. Eight thermally injured male patients, ages 18-37, with a range of total body surface burn of 60-90%, mean 70.2%, were examined 72-96 hours postburn. Patient mortality was 75%. Data are presented as \ln mean $\text{CPM}/10^6$ cells \pm 95% confidence limits.

	1*-C-Glucose		6*-C-Glucose	
	w/o Latex	w/Latex	w/o Latex	w/Latex
Control	5.5853 ± 0.1306	7.3800 ± 0.0851	2.4784 ± 0.1292	4.4244 ± 0.1544
Burn	4.6115 ± 0.3678	6.6893 ± 3355	1.3668 ± 0.3167	2.5557 ± 0.4795

Significance testing showed that cells from patients had reduced $^{14}\text{CO}_2$ production under all tested conditions ($p < 0.001$). This constant decrease

in oxidative metabolism of glucose by granulocytes following thermal injury may be a basis for the leukocyte dysfunctions previously demonstrated in like patient populations.

Burns
Leukocytes
Glucose oxidation
Latex phagocytosis

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OG 6953	76 10 01	DD-DR&E(AR)636	
3. DATE OF REV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY CTRY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF DISSEM
75 07 01	D. CHANGE	U	U	LA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY		61101A	3A161101A91C	00		084	
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Echocardiographic Evaluation of Left Ventricular Performance in the Severely Burned Military Population (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 06		Cont		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER:				76		.9	
c. TYPE:				77		.2	
d. KIND OF AWARD:				77		.5	
e. CUM. AMT.						25	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., COL, MC				NAME: James F. Dorethy, MAJ, MC			
TELEPHONE: 512-221-2720				TELEPHONE 512-221-5712			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME			
				NAME			
23. (U) To evaluate left ventricular function in the thermally injured patient, especially in postburn shock phase. To evaluate the hypothesis that myocardial depression is a direct consequence of severe thermal injury.							
24. (U) Serial left ventricular performance profiles will be derived echocardiographically and correlated to the clinical state of the patient.							
25. (U) 76 06 - 76 09 Due to delayed equipment delivery and subsequent calibration serial patient studies have just been initiated. The instrument has been used to assess the configuration of cardiac valves in patients suspected at having bacterial endocarditis. No valvular vegetations were identified by echocardiography in 15 patients with blood cultures positive for Staphylococcus aureus and in the four of these patients who died autopsy examination revealed normal cardiac values.							

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR PERFORMANCE IN THE SEVERELY BURNED MILITARY POPULATION

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

James F. Dorethy, M.D., Major, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR PERFORMANCE IN THE SEVERELY BURNED MILITARY POPULATION

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: James F. Dorethy, M.D., Major, MC

Reports Control Symbol MEDDH-288(R1)

Complex cardiovascular interactions following acute thermal injury have been studied by indirect methods of myocardial performance. Myocardial depression has been implicated as an etiology for decreased cardiac output during the early postburn period. No direct measurements of left ventricular performance have been reported.

This study proposes to utilize sensitive echocardiographic measurements of LV internal dimensions to evaluate postburn myocardial contractility. Due to delayed equipment delivery and subsequent calibration, only two serial studies have been carried out in the early resuscitative phase. Fourteen other patients have been studied serially for possible endocarditis. Two patients have been evaluated during septic shock.

No specific conclusions can be drawn from this small group of patients. However, this noninvasive technique appears feasible for serial studies of LV performance post-thermal injury.

Resuscitation
Burned soldiers
Echocardiography
Left ventricular function
Postburn shock

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMRY ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DES'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 10 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	61102A	3A161102B71P	02	070			
B. CONTRIBUTING							
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a							
(U) Renal Function in the Burned Soldier. I. Histology (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 01		76 09		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PREVIOUS		B. FUNDS (in thousands)	
B. NUMBER: ^a				76		.5	
C. TYPE:				FISCAL YEAR		22	
D. KIND OF AWARD				CURRENT		3	
E. AMOUNT:							
F. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a US Army Institute of Surgical Research				NAME: ^a US Army Institute of Surgical Research			
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RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: ^a William D. Myers, LTC, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-6532			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Thomas Rizzo Jr, MAJ, MC			
				NAME: Richard H. Merrill, LTC, MC DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Glomerulus; (U) Scanning EM; (U) Transmission EM; (U) Burn; (U) Azotemia; (U) Humans							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede rest of each with Security Classification Code.)							
23. (U) To identify electronmicroscopic glomerular pathology postburn and relate it to renal function changes in burned soldiers.							
24. (U) All patients in the burn unit with immediate postmortem authorization will undergo within one hour postmortem percutaneous needle biopsy of kidney. Tissue will be examined by light, electron, and immunofluorescent microscopy. Premortem renal function will be monitored by urinalysis, BUN, serum creatinine, urine creatinine, urine sodium, and urine potassium. Patients will be grouped by serum creatinine and pattern of function (6p.I:Cr less than 1.2; 6p.II:Cr more than 1.2; A:prerenal; B:renal). Clinical course noting type of burn, crush injury, resuscitation, coagulopathy, shock, sepsis; hypoxia, and medications will be recorded for future correlation.							
25. (U) 75 07 - 76 09 Twenty-five patients have been studied. Ten specimens were processed for immunoglobulin deposition and were interpreted as having no immunoglobulins present. Seventeen had interpretable transmission EM findings. The transmission EM findings included subendothelial rarefaction, segmental foot process fusion, increased mesangial matrix, and subendothelial dense deposits. No correlation could be found between renal function and EM alterations. Presently an effort is being made to correlate renal function with surface alterations demonstrated by scanning EM.							

^aAvailable to contractors upon contractor's approval

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498B 1 MAR 69 FOR ARMY USE ARE OBSOLETE

TERMINATION

**PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE**

REPORT TITLE: RENAL FUNCTION IN THE BURNED SOLDIER. I HISTOLOGY

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators

**William D. Myers, MD, Lieutenant Colonel, MC
Thomas Rizzo, Jr., MD, Major, MC
Richard H. Merrill, MD, Lieutenant Colonel, MC
John McPhaul, MD, Colonel, MC**

Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF
MILITARY MEDICINE

REPORT TITLE: RENAL FUNCTION IN THE BURNED SOLDIER.
I. HISTOLOGY

U. S. Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: William D. Myers, MD, Lieutenant Colonel, MC
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Reports Control Symbol MEDDH-288(R)

The purpose of this study was to determine if fibrin disposition immune complex disease, or toxic basement membrane alteration could explain the azotemia seen in the thermally injured patient. To answer this question patients underwent needle biopsy of the kidney within one hour of death and the tissue was submitted for light, electron, and immunofluorescent microscopy. Of the 25 samples 17 were adequate for electronmicroscopy, and 9 were adequate for immunofluorescent microscopy. Electronmicroscopy revealed spreading of lamina rara interna in 15, atypical subendothelial dense deposits in 5, increased mesangial matrix in 9, and focal foot process fusion in 7. Immunofluorescent microscopy of IgG, IgM, IgA, fibrinogen, and complement was negative in 8. One patient exhibited 1+ linear staining of uneven intensity from glomerulus to glomerulus of IgG, and trace to 1+ mesangial streaking of Complement (B,C). Histopathologic findings could not be correlated with serum creatinine levels in these patients.

Most electronmicroscopic alterations were considered to be due to early autolysis. There was no evidence that fibrin or immune complex deposition or basement membrane alteration was responsible for the altered renal function.

Glomerulus
Scanning EM
Transmission EM
Burn
Azotemia
Humans

RENAL FUNCTION IN THE BURN PATIENT: I. HISTOLOGY

The incidence of renal insufficiency following thermal injury has decreased with improved initial fluid resuscitation. However, even with adequate resuscitation, azotemia, which progresses to marked renal insufficiency, is occasionally seen. The typical pattern is that of a "prerenal" insufficiency manifested by a BUN/creatinine ratio greater than 10, urine urea nitrogen/serum urea nitrogen or urine creatinine/serum creatinine ratio greater than 20, urine specific gravity greater than 1.015, and a low excretion of sodium with a high excretion of potassium. The tubules continue to perform maximally as glomerular filtration progressively declines, revealing a glomerulotubular imbalance.

Graber and Sevitt² observed occasional lipid deposits in glomeruli and areas of tubular necrosis in the thermally injured patient. However, in spite of microscopic pathology, the tubules were able to reabsorb sodium and excrete potassium normally. It may be that the reduction in urine flow allows the tubules to reclaim most sodium from the slowly moving filtrate. A block in plasma flow from the glomerulus to the tubule could explain the clinical findings. Graber and Sevitt described multiple fine droplets of fat within the glomerular tufts and possibly within the endothelium and epithelium. However, no electron microscopy was performed. Tissue submitted from ISR autopsy specimens in the past for electron microscopy and immunofluorescent microscopy have been unsatisfactory for detailed study. Tissue obtained within one hour of death will hopefully provide interpretable study material.

This study will attempt to identify any electronmicroscopic glomerular pathology associated with normal and abnormal renal function in the thermally injured patient. All patients with immediate post-mortem authorization will undergo within one hour postmortem percutaneous needle biopsy of the kidney. Tissue will be examined by light and electron microscopy at the ISR Lab. Renal tissue will also be rapidly frozen and sent to Dr. McPhaul at Wilford Hall Air Force Hospital for immunofluorescent microscopy to determine the presence of IgG, IgA, fibrin, and complement. Renal function will be monitored by urinalysis, BUN, serum creatinine, urine creatinine, urine sodium, and urine potassium. Patients will be grouped by serum creatinine and pattern of function (Group I: Cr less than 1.2; Group II: Cr greater than 1.2; A: prerenal; B: renal). Clinical Course, noting time of burn,

1. Moncrief JA: Burns. New Eng J Med 288: 444, 1973.

2. Graber IG, Sevitt S: Renal function in burned patients and its relationship to morphological changes. J Clin Path 12: 25, 1959.

crush injury, resuscitation, coagulopathy, shock, sepsis, hypoxia, and medications will be recorded for future correlation.

RESULTS

Twenty-five patients were biopsied up to two hours after death. Of the twenty-five patients, only seventeen had interpretable electronmicroscopic findings. The electronmicroscopic glomerular alterations observed were subendothelial rarefaction in sixteen patients, segmental foot process fusion in eleven patients, increased mesangial matrix in ten patients, and subendothelial dense deposits in five patients. Of the eight uninterpretable specimens, four contained no glomeruli, two were mixed, and two were lost.

Of the twenty-five patients biopsied only ten had adequate samples for immunofluorescence microscopy. Nine specimens failed to demonstrate immunofluorescence to IgG, IgM, IgA, complement, or fibrinogen. One patient exhibited 1+ linear staining of uneven intensity from glomerulus to glomerulus of IgG, and trace to 1+ mesangial streaking complement (B₁C).

Table I summarizes relationships between electron microscopy, immunofluorescent microscopy, urine fibrin split products, maximum serum creatinine, burn size, and recognized nephrotoxins.

DISCUSSION

Several years ago Graber and Sevitt² addressed the relationship between renal histopathology and function. Tubular necrosis was observed in patients with normal renal function, and normal tubules were observed in patients with clinical renal failure. They concluded that there was little correlation between renal tubule histology and function, but suggested that a pretubular anatomic defect might explain the discrepancy. This study examined the relationship of renal function to glomerular histology.

Our clinical observations suggested that the renal insufficiency observed in the burn population was of the "prerenal" type. This was suggested by a BUN rising out of proportion to creatinine, very low urinary sodium excretion, very high urinary potassium excretion, UUN/BUN and Ucr/Scr ratio greater than 20. Such a pattern is compatible with glomerular tubular imbalance i.e., decreasing filtration in the presence of good tubular function. Mechanical blocking of flow from the blood side to the urine side of the capillary loop as in acute glomerulitis could give this functional pattern.

This study found no significant immune deposits in the glomeruli thus ruling out antigen-antibody type damage. Fibrin was also absent on electron and light microscopy. However, all most all glomerular capillary loops demonstrated some abnormality on electron microscopy, but none of these abnormalities could be correlated with level of serum creatinine. These results suggest that there is no gross mechanical blockage of flow from the blood side to the Bowman's space side of the capillary loop by

TABLE I						
PT (EX P MORT)	EM	HEMATO	PSP	Cr.	BUN	TREATMENT
PK (60)	Suben. rare w/ DD	Neg	4:1	1.3	60	Cells, V
VP (60)	Suben. rare, Seg PP Fus.	Neg	16:1	5.9	73	Cells
TD (60)	Suben. rare, Seg PP Fus	Neg	8:1	3.5 ^{BS}	66	Cells
WS (60)	Suben. rare, Seg PP Fus, M	U	1:1	2.1 ^{BS}	60	G
MG (60)	Suben. rare, Seg PP Fus, M	Neg	8:1	4.6 ^{BS}	66	Cells, G
TF (60)	Suben. rare, Seg PP Fus, M	U	8:1	1.6	54	Cells
DK (60)	Suben. rare Seg PP Fus, M	U		2.3	76	
JC (60)	Suben. rare, Seg PP Fus, M	U		2.3 ^{BS}	62	Cells
WR (60)	Suben. rare, Seg PP, Fus, M		4:1	0.9	62	Cells
KSH (45)		+	+	2.6	68	Cells G.
RM (90)	Suben. rare, Seg PP Fus, M	Neg	8:1	6.0 ^{BS}	37	Cells G
DS (120)	BM, DM, Seg PP Fus	Neg	1:1	2.1 ^{BS}	66	Cells
CL (90)	Mixed	U	13:1	BUN30	60	
LAD (30)	Mixed	U	1:1	0.8	67	Cells, G
LJ (120)	NG	U	8:1	3	75	
CS (30)	NG	U	8:1	6.2 ^{BS}	67	Cells V
RD (90)	Suben. rare, M	U	8:1	0.6	50	
DC (120)	NG	U	1:1	3.6 ^{BS}	51	G
RS (120)	NG	U	1:1	3.5 ^{BS}	70	Cells
EB	Suben. rare w/DD		4:1	6 ^{BS}	50	Cells, G
WC	Suben. rare w/DD, Seg PP Fus, M		8:1	1.2	33	Cells, V
AV				6.3 ^{BS}	67	Cells, G
DB (60)	Suben. rare, M	Neg		0.7	79	
DB (60)	Suben. rare (Minimal)	Neg		1.3	70	
WS (60)	Suben. rare w/DD	Neg		4.4 ^{ATN}	36	Cells

KEY TO TABLE I

Suben. rare - Subendothelial rarefaction	DD - Dense deposit
Seg. PP Fus - Segmental Foot Process Fusion	M - Mesangial hypertrophy
BM - Thickened Basement Membrane	DM - Diabetes Mellitus
Mixed - Specimens Intermixed	NG - No glomeruli
U - Unsatisfactory	BS - Burn Syndrome (Prerenal)
ATN - Acute Tubular Necrosis	Cells - Cellulitis
V - Vancomycin	G - Gentamycin
PSP - Fibrin Split Products	

immunoglobulins or fibrin. Although electronmicroscopic alterations could account for reduced filtration, it is unlikely since serum creatinine varied from 0.7 to 8.0 regardless of electronmicroscopic alterations. The histologic findings in this study may represent only postmortem autolysis.

Recently scanning electronmicroscopy has revealed glomerular surface alterations in acute renal failure models³, again raising the question of relationship between histologic and functional alteration. These surface changes and their relationship to renal function need to be further evaluated. Such an investigation is presently underway.

PRESENTATIONS AND/OR PUBLICATION

None

3. Cox JW, et al: Studies on the mechanism of oliguria in a model of unilateral acute renal failure. J. Clin. Invest. 53: 6, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OG 6961	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DWSN INSTR ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS	
76 10 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO / CODES ^a		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		61102A	3A161102B71P	01	021		
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a							
(U) Localization of Infection Within the Urinary Tract of Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 11		76 09		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE		EXPIRATION		PRECEDING		b. FUNDS (in thousands)	
b. NUMBER ^a				76		.4	
c. TYPE		d. AMOUNT.		71		15	
e. KIND OF AWARD		f. CUM. AMT.		CURRENT		2	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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TELEPHONE. 512-221-2720				TELEPHONE 512-221-6532			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Dr. Gersh			
				NAME Thomas Rizzo, MAJ, MC DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Yeast; (U) Bacteria; (U) Immunofluorescence; (U) Urinary tract; (U) Humans							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
23. (U) To determine the degree of spread of infection from the lower to the upper urinary tract in burned soldiers.							
24. (U) Patients with indwelling bladder catheters will have urine examined daily for bacteria and/or yeast. Those patients found to have organisms in the urine will have their urine processed to determine if the organisms are coated with immunoglobulin. Patients found to have organisms which are coated with immunoglobulin will have urine sent to bacteriology for culture and sensitivity testing so that appropriate antibiotic therapy can be instituted. Those patients coming to post mortem examination will have detailed examination of the urinary tract for organism invasion.							
25. (U) 75 11 - 76 09 Twenty patients have been evaluated to date. Of the five with bacteruria none were demonstrated to have immunoglobulin coating, suggesting only bladder infection. Of the fifteen with candiduria, only those with hyphae were found to have immunofluorescence. No correlation has been made between anatomic site of infection by postmortem examination and yeast immunoglobulin coating. No conclusions can be drawn until more patients are studied.							

^aAvailable to contractors upon originator's approval.

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1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 66
AND 1498 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

TERMINATION

**PROJECT NO. 3A161102B71P-01, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE**

REPORT TITLE: LOCALIZATION OF INFECTION WITHIN THE URINARY TRACT

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators

**William D. Myers, M.D., Lieutenant Colonel, MC
Harvey A. Gersh, M.D., Major, MC
Thomas Rizzo, Jr., M.D., Major, MC
Marvin Forland, M.D.
V. Thomas, PhD**

Reports Control Symbol MEDDH-288(RI)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-01 BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: LOCALIZATION OF INFECTION WITHIN THE URINARY TRACT

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: William D. Myers, M.D., Lieutenant Colonel, MC
Harvey A. Gersh, M.D., Major, MC
Thomas Rizzo, Jr., M.D., Major, MC
Marvin Forland, M.D.
V. Thomas, PhD

Report Control Symbol MEDDH-288 (RI)

Budding yeast and hyphae are frequently observed in the urine of thermally injured patients requiring prolonged indwelling urinary bladder catheterization and systemic antibiotics. This investigation was designed to determine if yeast infections of the urinary tract can be localized by staining for immunoglobulin on the yeast, to determine what percent of catheterized patients develop upper tract disease, and to determine if appropriate therapy can reduce the incidence of sepsis by identifying upper tract disease early.

Twenty patients have been evaluated to date. Of the 5 with bacteriuria, none were demonstrated to have immunoglobulin coating suggesting no extra-bladder infection. Of the 25 with candiduria, only those with hyphae were found to have immunofluorescence. No correlation has been made between anatomic site of infection by postmortem examination and yeast immunoglobulin coating. No conclusions can be drawn until more patients are studied.

Bacteriuria
Immunoglobulin
Infection
Soldier
Yeast
Immunofluorescence

LOCALIZATION OF INFECTION WITHIN THE URINARY TRACT

Budding yeast and hyphae are frequently observed in the urine of thermally injured patients requiring prolonged indwelling urinary bladder catheterization and systemic antibiotics. Hyphae in the urine have been considered a sign of tissue invasion, rather than just a sign of contaminated urine in the bladder; however, if the infection is limited to the bladder, it can be managed by local irrigation of the bladder with 5 flucytosine or amphotericin, thus reducing the toxicity of systemic therapy². If the kidney was infected, local bladder irrigation would not be sufficient. Recently, it has been reported that bacteriuria can be localized as coming from the kidney or bladder by staining the urine for bacteria coated with immunoglobulins^{3,4}. Those bacteria originating in the kidney would be immunoglobulin coated and those coming from the bladder should not be coated. However, one must be careful when the patient is a male because prostatitis can also coat bacteria; therefore, "everything that coats" is not always of kidney origin. If yeast in the urine, originating from areas other than the bladder, were also coated with an immunoglobulin, then this could be used to determine if the patient should be managed with systemic antifungal therapy in addition to local therapy.

Previous investigators at this institute have reported that once the urinary catheter is removed, bacteriuria frequently resolves without therapy.⁵ Those patients who did not have spontaneous resolution of bacteriuria may have had upper tract infections requiring further management. By staining the bacteria in the urine for immunoglobulin, one can identify this group and manage them appropriately.

This investigation was designed to answer the following questions:

1. Can yeast infections of the urinary tract be localized by staining for immunoglobulin on the yeast?
2. What percentage of catheterized patients develop upper tract disease?
3. Can appropriate therapy reduce the incidence of sepsis of identifying upper tract disease early?

1. Kosinn PJ, Taschdjian CL: Enteric Candidiasis: diagnosis and clinical considerations. *Pediatrics* 30: 71, 1962.

2. Wise GJ et al: Candidal cystitis. *JAMA* 224: 1636, 1973.

3. Thomas V, et al: Antibody-coated bacteria in the urine and the sites of urinary tract infection. *New Eng J Med* 290: 558, 1974.

4. Thomas V, et al: Immunoglobulin levels and antibody-coated bacteria in urines from patients with urinary tract infections. *Pros Soc Exper Biol Med* 148: 1198, 1975.

5. Reckler JM: Urinary tract infections in burned patients. *USAISR Annual Research Progress Report*, June 1971, pg 11.

4. After appropriate therapy, does coated or uncoated bacteriuria recur?

METHODS

All patients found to have yeast, by microscopic examination of the urine, will have urine set aside for immunofluorescence. Those patients coming to postmortem examination will be specifically observed for yeast infection of the kidney, ureter, bladder wall, and prostate, as well as other areas of tissue invasion. The pathologic findings will be correlated with the immunofluorescence of the organisms in the urine.

All patients with indwelling catheters will have urine screened for bacteria which, in turn, will be subjected to immunofluorescence. Clean catch-midstream urine will also be screened after the urinary bladder catheter is removed. Those patients found to have "coated" bacteriuria, as well as those with "noncoated" bacteriuria found 48 hours after removal of the urinary catheter, will be treated with the appropriate antibiotic, as determined by culture and sensitivity. Urine will be constantly screened after any therapy for urinary tract infection.

Staining of bacteria and yeast in the urine for immunoglobulin will be done by the technique described by Thomas, et al³

RESULTS

Twenty patients have been evaluated to date. Of the 5 with bacteriuria, none were demonstrated to have immunoglobulin coating suggesting no extrabladder infection. Of the 15 with candiduria, only those with hyphae were found to have positive immunofluorescence tests. No correlation has been made between anatomic site of infection by postmortem examination and yeast immunoglobulin coating. No conclusions can be drawn until more patients are studied.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL (DD DR&E(AR)456)	
3 DATE PREV. SUMMRY	4 KIND OF SUMMARY	5 SUMMARY SCTY ^a	6 WORK SECURITY ^a	7 REGRADING ^a	8A DIS'TN INSTR'N	8B SPECIFIC DATA CONTRACTOR ACCESS	9 LEVEL OF SUM
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A WORK UNIT
10 NO. CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61102A	3A161102B71P	02	069			
b. CONTRIBUTING							
c. CONTRIBUTING							
11 TITLE (Precede with Security Classification Code) ^a (U) To Determine the Role of the Prostaglandins (PG) in the Response to Volume Expansion in the Dog--A Model of Changes in Injured Troops (44)							
12 SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13 START DATE		14 ESTIMATED COMPLETION DATE		15 FUNDING AGENCY		16 PERFORMANCE METHOD	
75 02		76 09		DA		C. In-House	
17 CONTRACT/GRANT Not Applicable				18 RESOURCES ESTIMATE		19 PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PREESTIM		b. FUNDS (in thousands)	
b. NUMBER ^a				76		.4	
c. TYPE				FISCAL YEAR		13	
d. KIND OF AWARD				CURRENCY			
19 RESPONSIBLE DOD ORGANIZATION				20 PERFORMING ORGANIZATION			
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research			
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TELEPHONE 512-221-2720				TELEPHONE 512-221-4698			
21 GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Richard H. Merrill, LTC, MC			
				NAME			
22 KEYWORDS (Precede EACH with Security Classification Code) (U) Animal; (U) Renal; (U) Autoregulation; (U) Dogs; (U) Prostaglandins; (U) Volume expansion							
23 TECHNICAL OBJECTIVE, 24 APPROACH, 25. PROGRAM (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) In the established dog model of volume expansion, responses in adaptation and autoregulation occur. Renal prostaglandins are thought to play a role in this autoregulation. The objective in this study is to observe response to volume expansion in the absence of prostaglandins as a model of changes in injured troops.</p> <p>24. (U) Volume expansion in the dog model will be accomplished with 7.5% body weight expansion with Ringer's Lactate (RL). For comparison, 6.5ml/kg body weight of hyperoncotic albumin (HA) will be used to study the difference between vascular and interstitial expansion (RL) and vascular expansion alone (HA). The role of prostaglandin (PG) in a response to volume expansion will be tested by inhibiting PG synthesis with IV Indomethacin 2mg/kg body weight.</p> <p>25. (U) 75 07 - 76 09 The study was designed to observe animals response to RL and HA before and after PG inhibition. The data show no effect of PG inhibition on baseline studies and in no way alter the response to volume expansion with either RL or HA.</p>							

^a Available to contractors upon originator's approval

TERMINATION

**PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF
MILITARY MEDICINE - SURGICAL
PATHOLOGY**

**REPORT TITLE: TO DETERMINE THE ROLE OF THE PROSTAGLANDINS (PG)
IN THE RESPONSE TO VOLUME EXPANSION IN THE DOG -
A MODEL OF CHANGES IN INJURED TROOPS**

**U S ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 July 1975 - 30 September 1976

Investigators

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ABSTRACT

PROJECT NO. 3A161102B71R-02, BASIC RESEARCH IN SUPPORT OF
MILITARY MEDICINE- SURGICAL
PATHOLOGY

REPORT TITLE: TO DETERMINE THE ROLE OF THE PROSTAGLANDINS IN THE
RESPONSE TO VOLUME EXPANSION IN THE DOG - A MODEL
OF CHANGES IN INJURED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: David B. Olin, MD, Major, MC
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Isotonic volume expansion in man and animals results in slight changes in glomerular filtration rate (GFR) and renal blood flow, but dramatically increases urinary sodium excretion and fractional excretion of sodium (FE_{Na}). The factors leading to this response remain unclear. Physical factors (Starling forces) may account for the response by increasing the proximal tubular backleak of sodium. The concept of a hormonal factor which may be natriuretic (III factor) arose from work of De Wardner in which cross perfused dogs receiving blood from volume expanded animals underwent natriuresis despite the fact that they themselves were not volume expanded. The character and mechanisms of release of this factor remained to be defined. Work from this laboratory and others has shown prostaglandins E (PGE) to have natriuretic properties when infused into the renal artery. This effect seems to be mediated by vasodilation, as filtration decreases and proximal tubular reabsorption of sodium, glucose, and bicarbonate are decreased. The physiologic significance of intra-arterial infusions of PGE have been challenged due to the low levels of PGE in the circulation and its site of synthesis has resulted in increased urinary sodium. To further test the role of PG in renal auto-regulation, PG synthesis was inhibited by Indomethacin. To observe the role of PGE in volume expansion Ringer's Lactate (RL) was used. Further studies were performed with hyperoncotic albumin (HA) to observe any variation in PG's role in the presence of interstitial volume contraction which results with hyperoncotic albumin.

Animal
Renal
Autoregulation
Dogs
Prostaglandins
Volume expansion

TO DETERMINE THE ROLE OF THE PROSTAGLANDINS IN THE RESPONSE TO VOLUME EXPANSION IN THE DOG - A MODEL OF CHANGES IN INJURED TROOPS

Isotonic volume expansion in man and animals results in slight changes in glomerular filtration rate (GFR) and renal blood flow, but a dramatic increase in urinary sodium excretion and fractional excretion of sodium (FE_{Na}). The factors leading to this response remain unclear. Physical factors (Starling Forces) may account for the response by increasing the proximal tubular backleak of sodium. The concept of a hormonal factor which may be natriuretic (III Factor) arose from work of De Wardner in which cross perfused dogs receiving blood from volume expanded animals underwent natriuresis despite the fact that they themselves were not volume expanded. The character and mechanisms of release of this factor remained to be defined. Work from this laboratory and others has shown prostaglandins E (PGE) to have natriuretic properties when infused into the renal artery (1,2,3). This effect seems to be mediated by vasodilation, as filtration fraction decreases and proximal tubular reabsorption of sodium glucose, and bicarbonate are decreased. The physiologic significance of intra-arterial infusions of PGE have been challenged due to the low levels of PG in the circulation and its site of synthesis being in the renal medulla. Infusion of arachidonic acid (immediate precursor of PGE in the kidney) into the renal artery, indirectly increasing renal PG synthesis have resulted in increased urinary sodium. To further test the role of PG in renal auto-regulation, PG synthesis was inhibited by indomethacin. To observe the role of PGE in volume expansion Ringer's lactate (RL) was used. Further studies were performed with hyperoncotic albumin (HA) to observe any variation in PG's role in the presence of interstitial volume contraction which results with hyperoncotic albumin.

METHODS

Studies were performed in the Metabolic Branch, USAISR. Mongrel dogs weighing between 8 and 20 kilograms were utilized. The animals were given nothing by mouth after 6 pm of the day prior to surgery. The animals were anesthetized with sodium pentothal and respirations were maintained by a Byrd respirator. Supplemental doses of sodium pentothal were given as needed. Femoral vessels were isolated for monitoring arterial pressure, blood sampling, and intravenous infusions. Both ureters were isolated through a small suprapubic incision. In a few animals, a left flank incision

1. Martinez-Maldonado M, Tsparas N, Eknayan G, Suki W: Renal actions of prostaglandins: Comparison with acetylcholine and volume expansion. *Am J Physio* 222: 1147, 1972.

2. Johnston H, Herzog J, Lauler D: Effect of prostaglandin E_1 on renal hemodynamics sodium and water excretion. *Am J Physio* 213: 939, 1967.

3. Gross J, Barlter F: Effects of prostaglandins E_1 , A_1 , and F_2 x on renal handling of salt and water. *Am J Physio* 225: 218, 1973.

was utilized to isolate the renal vein for determinations of para-aminohippurate (PAH) extraction.

all animals were given 20 to 40 ml/kg 5% Dextrose in water intravenously in order to establish a water diuresis during control periods. All urine output was replaced with 5% Dextrose in water. Loading doses of Inulin 15 mg/Kg and PAH 8 mg/Kg were given and infusion of Inulin and PAH was started. The animal was allowed 30 minutes to recover from surgery and then baseline control periods of 10 to 15 minutes each were performed.

After the control periods, the animals were treated in the following manner:

Group I - Ringer's lactate alone - 7.5% body weight volume expansion

Group II - Indomethacin 2 mg/Kg body weight

Group III - Hyperoncotic albumin - 6.5 ml/Kg body weight

The response was then observed, measuring clearance of Inulin, clearance of PAH, urinary sodium excretion, fractional excretion of sodium, osmolar clearance, clearance of free water, blood pressure and hematocrit. In addition to the above data, serum and urinary calcium, phosphorus, potassium, and chloride were obtained. After the response was observed, the following was done to each animal group:

Group I - Indomethacin 2 mg/Kg IV

Group II - Sub group A - Ringer's lactate 7.5% body weight

Sub group B - Hyperoncotic albumin 6.5 ml/ Kg

Group III - Indomethacin 2 mg/kg IV

Repeat data was collected for comparison with the previous data for clearance of Inulin, clearance C_{PAH} , urinary sodium excretion ($U_{Na} V$)

fractional excretion of sodium, (FE_{Na}) clearance of Osmoles, (C_{OSM}) free

water clearance, (C_{H_2O}), blood pressure and hematocrit. In addition, 2

to three animals were used as controls for both Ringer's lactate volume expansion and hyperoncotic albumin expansion, and received no Indomethacin.

LABORATORY METHODS

Laboratory tests were performed in the Biochemistry Laboratory of the Institute of Surgical Research. All tests were performed on both serum and urine utilizing the following techniques. Sodium and potassium were done under routine techniques on the flamephotometer. Chloride determinations were performed on the Buchler-Cotlove chloridimeter utilizing standard techniques. Serum and urine values for Inulin, PAH, calcium, phosphorus, creatinine, and blood urea nitrogen were all performed on the autoanalyzer using adaptation of standard methods.

These techniques were modified by the personnel of the institute of Surgical Research Laboratory for utilization in this study. Calculations of urinary sodium excretion using urine sodium concentration and volume as well as fractional excretion of sodium utilizing urinary sodium excretion and glomerular filtration rate as estimated by the clearance of Inulin were all performed utilizing standard physiologic formulas. Clearance data for osmolality and the calculations of free water clearance were made using the formula $V = C_{\text{osm}} + C_{\text{H}_2\text{O}}$.

All studies utilizing Indomethacin were performed 30 minutes after the intravenous dosage was administered (4).

The animals were sacrificed at the end of the study.

The data represents single observations in each animal in each condition studied. Paired T analysis was performed on these data. Mean and standard deviations of each group were determined for the purposes of comparison.

RESULTS

Results from animals in Group I receiving 7.5% body weight volume expansion with RL are in Table I. Following volume expansion there were significant increases in urine flow, FE_{Na} , C_{osm} , and $C_{\text{H}_2\text{O}}$. After Indomethacin

was given there were no changes in any of these parameters.

Group II animals were given 2 mg/kg Indomethacin. There was no change in any parameter (Table I). Following the Indomethacin, Group IIA received 7.5% body weight volume expansion with RL. There was significant change in urine flow, FE_{Na} , and $C_{\text{H}_2\text{O}}$ with slight but not significant increases in C_{osm} (Table II)

The changes were similar to those of Group I. Group IIB received 65ml/kg 1.4% volume expansion following the Indomethacin (Table III). These animals also showed no change in any parameter following Indomethacin. After HA there were significant increase in urine flow, FE_{Na} , and $C_{\text{H}_2\text{O}}$. These changes were similar

to Group III animals. Group III animals received 6.5ml/100g HA (Table IV). The HA resulted in significant increase in urine flow and $C_{\text{H}_2\text{O}}$ yet no significant

increase in FE_{Na} . Indomethacin resulted in no change in any parameter other than slight decrease in FE_{Na} ($p = .05$).

4. Vane JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature (New Biology)* 231: 232, 1971.

TABLE I

RINGERS LACTATE (4)

	<u>CONTROL</u>	<u>EXPERIMENTAL</u>	<u>EXP + INDO</u>
FLOW	1.1±.99	6.2±2.26 P=0.025	6.57±1.46 NS
GFR	52.77±15	74±31.4 NS	78.8±31.4 NS
FE _{Na}	0.7±.85	3.32±1.82 P=0.01	4.37±3.0 NS
C _{osm}	1.73±.73	3.38±.65 P<.025	3.95±1.22 NS
C _{H2O}	-.64±.73	2.78±2.0 P<.025	2.59±7.49 NS
BP	129±12	114±8 NS	139±26 NS
HCT	44(1)	32(1)	

TABLE II

	<u>CONTROL + INDO</u>	<u>RL</u>
FLOW	2.49±1.8	9.82±4.21<.025
GFR	49±25	55.18±25 NS
FENa	.92±.23	4.8±1.36<.01
Cosm	1.48±.89	3.75±2.68 NS
CH ₂ O	.8±1.28	5.7±2.93<.05
BP	135±12	136±11 NS

TABLE III

	<u>CONTROL</u>	<u>CONTROL + INDO</u>	<u>HA</u>
FLOW	1.94	2.23 NS	11.9<.001
GFR	60.77	60.51 NS	61.65 NS
FENa	.47	.99	5.58<.01
C _{osm}	1.8	2.0 NS	4.23 NS
C _{H₂O}	-.69	1.0	7.45<.05
BP	174	174 NS	174 NS

TABLE IV

SALT POOR ALBUMIN (7)

	<u>CONTROL</u>	<u>EXPERIMENTAL</u>	<u>EXP + INDO</u>
Flow	2.75±3.3	9.36±4.85 P<0.001	9.14±5.6 NS
GFR	62±11.7	70.6±21.2 NS	72.42±19 NS
FE _{Na}	1.39±1.76	2.6±2.2 NS	2.14±1.93<.05
C _{osm}	2.71±1.6	3.95±2.9 NS	3.63±3.19 NS
C _{H₂O}	.74±2.8	6.68±3.12 P<0.005	7.2±2.74 NS
BP	154±27	152±23.9 NS	161.5±23 NS
HCl ⁻	32.9±5.6	25.6±3.6 P<0.001	

COMMENTS

Volume expansion with Ringer's lactate results in both interstitial as well as intravascular volume expansion. Our data suggest that prostaglandin inhibition results in no change from control period data and does not affect response to 7.5% body weight volume expansion with Ringer's lactate. This lack of response was observed both before volume expansion and 30 to 45 minutes after volume expansion. This observation has also been made by Kirschenbaum et al (5).

Further studies in unanesthetized animals suggest that prostaglandin inhibition leads to a natriuresis (6). This observation is contrary to previous observations in anesthetized animals and may be important. Sonnenburg and Stein have suggested that since prostaglandins are synthesized in the medulla of the kidney, they may act locally (6,7). This eliminates the need to postulate a circulating hormone like effect of the prostaglandins. The theory can be stated that interstitial volume contraction leads to an increase in prostaglandin synthesis thus, increasing collecting duct sodium reabsorption. In interstitial expansion, the opposite would occur.

During volume expansion with hyperoncotic albumin, similar degrees of intravascular volume expansion (judged by plasma volume measurements and changes in hematocrit) do not result in comparable natriuresis as with Ringer's lactate. (8,9) The lesser natriuresis is seen despite an increase in volume in urine and an increase in free water clearance suggesting similar distal delivery of sodium. Hyperoncotic albumin results in interstitial volume contraction. Thus, prostaglandin synthesis could be stimulated and distal tubule or collecting duct sodium reabsorption increased to explain the observation of a lesser degree of natriuresis. The present data show no change in fractional excretion of sodium, urinary sodium excretion, free water clearance, or osmolar clearance when prostaglandin inhibition was performed both prior to volume expansion as well as after volume expansion. These studies were performed in anesthetized animals to confirm these observations. The role of renal prostaglandin in renal autoregulation is still unclear. Further testing needs to be performed utilizing antagonists and blockers of prostaglandin synthesis other than Indomethacin.

5. Kirschenbaum M, Stein J: The effect of inhibition of prostaglandin synthesis on urinary sodium excretion in the conscious dog. *JCI* 57: 517, 1976.

6. Stein J, Kirschenbaum M, Bay W, Osgood R, Fenis T: Role of collecting duct in the regulation of sodium balance. *Cir Res Supp* 36 & 37, 1-119; 1975.

7. Sonnenburg H: Renal response to blood volume expansion: Distal tubular function and urinary excretion. *Am J Physio* 223: 916, 1972.

8. Levy M, Levinsky N: Proximal reabsorption and intrarenal pressure during colloid infusions in the dog. *Am J Physiol* 220: 415, 1971.

9. Howards SS, Davis BB, Knox FG, Wright FS, Berliner RW. Depression of fractional sodium reabsorption by the proximal tubule of the dog without sodium diuresis. *J Clin Invest* 47: 156

PUBLICATIONS AND/OR PRESENTATIONS

None

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1. Martinez-Maldonado M, Tsparas N, Eknoyan G, Suki W: Renal actions of prostaglandins: Comparison with acetylcholine and volume expansion. *Am J Physiol* 222: 1147, 1972.
2. Johnston H, Herzog J, Lauler D: Effect of prostaglandin E_1 on renal hemodynamics sodium and water excretion. *Am J Physiol* 213: 939, 1967.
3. Gross J, Barlter F: Effects of prostaglandins E_1 , A_1 , and F_{2x} on renal handling of salt and water. *Am J Physiol* 225: 218, 1973.
4. Vane J: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature (New Biology)* 231: 232, 1971.
5. Kirschenbaum M, Stein J: The effect of inhibition of prostaglandin synthesis on urinary sodium excretion in the conscious dog. *J. Clin Invest* 57: 571, 1976.
6. Stein J, Kirschenbaum M, Bay W, Osgood R, Fenis T: Role of collecting duct in the regulation of sodium balance. *Cir Res supp* 36 & 37, 1-119, 1975.
7. Sonnenburg H: Renal response to blood volume expansion: Distal tubular function and urinary excretion. *Am J Physiol* 223: 916, 1972.
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9. Howards S, Davis B, Knox F, Wright F and Berliner R: Reabsorption by the proximal tubule of the dog without sodium diuresis. *J Clin Invest* 47: 1561, 1968.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OF 6387	76 10 01	DD-DR&E(AR)636	
3 DATE PREV SUMRY	4 KIND OF SUMMARY	5 SUMMARY SCTY ^a	6 WORK SECURITY ^a	7 REGRADING ^a	8A DISTR INSTR ^a	8B SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10 NO /CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	61102A	3A161102B71P		02		068	
B. CONTRIBUTING							
C. CONTRIBUTING							
11 TITLE (Precede with Security Classification Code) ^a (U) To Determine Whether the Prostaglandins are Important in Development of Acute Renal Failure--A Rabbit Model of Renal Failure in Soldiers (44)							
12 SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13 START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16 PERFORMANCE METHOD	
75 02		76 09		DA		C. In-House	
17 CONTRACT/GRANT				18 RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
Not Applicable				PRECEDING			
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B. NUMBER ^a				76		10	
C. TYPE.				YEAR			
D. KIND OF AWARD				CURRENT			
E. AMOUNT:							
F. CUM. AMT.							
19 RESPONSIBLE DOD ORGANIZATION				20 PERFORMING ORGANIZATION			
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21 GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Richard H. Merrill, LTC, MC			
				NAME:			
22 KEYWORDS (Precede each with Security Classification Code)							
(U) Acute renal failure; (U) Gentamicin; (U) Prostaglandins; (U) Rabbits							
23 TECHNICAL OBJECTIVE, 24 APPROACH, 25. PROGRESS (Pursue individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To establish a model of Gentamicin nephrotoxicity simulating a cause of renal failure in injured soldiers.							
24. (U) New Zealand white rabbits will be utilized and given daily IM injections of Gentamicin to observe for two weeks whether nephrotoxicity can be established.							
25. (U) 75 07 - 76 09 New Zealand white rabbits maintained on regular diets and receiving 40mg/kg and 80 mg/kg IM Gentamicin suffered no ill effects and maintained stable serum electrolytes during two week study period. An additional study comparing effect of changing drinking water to NH ₄ Cl or NaCl and again followed for two weeks showed no effects other than mild decrease serum bicarbonate. New Zealand white rabbits tolerate massive doses of Gentamicin nephrotoxic. ∴ Thus, study of acute renal failure in this animal is not practical.							

*Available to contractors upon originator's approval

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TERMINATION

**PROJECT NO. 3A161102B71P-02 BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY**

**REPORT TITLE: TO DETERMINE WHETHER THE PROSTAGLANDINS ARE
IMPORTANT IN DEVELOPMENT OF ACUTE RENAL FAILURE
- A RABBIT MODEL OF RENAL FAILURE IN SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 July 1975 - 30 September 1976

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ABSTRACT

**PROJECT NO. 3A16112B71R-02 BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY**

**REPORT TITLE: TO DETERMINE WHETHER THE PROSTAGLANDINS ARE
IMPORTANT IN DEVELOPMENT OF ACUTE RENAL FAILURE -
A RABBIT MODEL OF RENAL FAILURE IN SOLDIERS**

**US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
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Period covered in this report: 1 July 1975 - 30 September 1976

**Investigators: David B. Olin, MD, Major, MC
Richard H. Merrill, MD, LTC, MC**

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The clinical observation of Gentamicin nephrotoxicity is well established. Patients receiving Gentamicin may experience transient renal insufficiency or frank renal failure. In the burn soldier, with a high risk of systemic infection, Gentamicin is a commonly used antibiotic. In an effort to further study the pathogenesis of Gentamicin nephrotoxic renal failure, an attempt was made to create an animal model. Previous studies utilizing the rat have been reported. The New Zealand white rabbit was chosen for the present investigation because of the larger size animal and the observation of high levels of circulating prostaglandins E and A (personal observation).

The observation of renal failure associated with aminoglycoside antibiotics is common, yet the mechanisms are not clear. The drug is filtered and reabsorbed in proximal and distal tubules. It is thought that renal failure results from the concentration of drugs in the tubular cell since volume depletion seems to increase reabsorption and thus predispose to renal failure. The rabbit model was proposed to evaluate the pathogenesis of renal failure associated with Gentamicin. Massive doses were utilized (10 to 20 times therapeutic dosage) to insure high dose exposure. Blood levels were measured at 4 to 8 hours and found to be in the 20 to 40 ug mg/ml range showing adequate absorption of the intramuscular injections. It is concluded that the rabbit is not a practical animal model to investigate Gentamicin induced renal failure, since it has not been possible to induce even modest renal impairment in this species. The mechanisms of handling of Gentamicin in the rabbit kidney were not investigated, but may be of interest in future investigations.

**Renal Failure
Gentamicin
Animal
Prostaglandins
Rabbits**

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6980	76 10 01	DD-DR&E(AR)636	
3 DATE PREV SUMRY	4 KIND OF SUMMARY	5 SUMMARY SCTY ^a	6 WORK SECURITY ^a	7 REGRADING ^a	8A DISB'N INST'N	8B SPECIFIC DATA - CONTRACTOR ACCESS	9 LEVEL OF SUM
75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10 NO / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA/ NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3A161102B71P		02		067	
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) The Effects of Calcium on the Renin-Angiotensin System- Use of an Animal Model of Hypertension in Military Personnel (44)							
12 SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13 START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16 PERFORMANCE METHOD	
74 01		76 09		DA		C. In-House	
17 CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PREVIOUS		b. FUNDS (in thousands)	
EXPIRATION				76		.4	
b. NUMBER ^a				FISCAL		7	
c. TYPE.				YEAR		CURRENT	
d. KIND OF AWARD				e. AMOUNT		f. CUM. AMT.	
19 RESPONSIBLE DOD ORGANIZATION				20 PERFORMING ORGANIZATION			
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21 GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Calcium; (U) Renin; (U) Angiotensin; (U) Hypertension; (U) Dogs							
23. TECHNICAL OBJECTIVE ^a 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To assess the role of calcium in the stimulation of the renin angiotensin system as related to hypertension in military personnel.</p> <p>24. (U) A series of dogs will be studied as the experimental model. Various concentrations of calcium will be infused directly into the renal artery, and the production of renin angiotensin will be measured by direct cannulation of the renal vein. The contralateral kidney will remain uninfused and will serve as a control.</p> <p>25. (U) 75 07 - 76 09 The experience with the lightly anesthetized animal model in investigating the effect of calcium on the renin angiotensin system does not allow firm conclusions to be drawn. Because the amount of preparatory surgery in this animal model is considerable, it has been impossible to stabilize the animal within a reasonable period of time for the experimental protocol. The glomerular filtration rate and renal blood flow have varied considerably within the control period and the results obtained could easily be explained by the instability of the animal during the immediate post operative period rather than by the effects of calcium infusion. The trend of renal research is now directed toward the study of the awake intact animal. We have returned to the difficult problem of creating a suitable intact animal model. Using newer techniques for protecting the kidneys during prolonged surgery and utilizing custom designed silastic catheters and fittings, some early success has been achieved in the creation of the awake intact dog model. It is to be emphasized that the time spent on the creation of the dog model is necessary and important since it will represent an important advance in future renal studies. It is anticipated that this model will form the basis of future experimentation from this laboratory.</p>							

^aAvailable to contractors upon originator's approval.

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TERMINATION

**PROJECT NO. 3A161102B71P-02 BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY**

**REPORT TITLE: THE EFFECTS OF CALCIUM ON THE RENIN-ANGIOTENSIN
SYSTEM - USE OF AN ANIMAL MODEL OF HYPERTENSION**

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MEDICINE - SURGICAL PATHOLOGY

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The experience with the lightly anesthetized animal model in investigating the effect of calcium on the renin angiotensin system does not allow firm conclusions to be drawn. Because the amount of preparatory surgery in this animal model is considerable, it has been impossible to stabilize the animal within a reasonable period of time for the experimental protocol. The glomerular filtration rate and renal blood flow have varied considerably within the control period and the results obtained could easily be explained by the instability of the animal during the immediate postoperative period rather than by the effects of calcium infusion. In addition it is clear from the recent Nephrology literature that many of the assumptions classically made concerning experiments in the anesthetized dog bear little physiologic significance. The trend of renal research is now directed toward the study of the awake intact animal. We have returned to the difficult problem of creating a suitable intact animal model which will be suitable for sophisticated experiments involved in this protocol and others to follow. Because of the requirement of multiple vessel cannulation as well as exteriorizing ureters, other extant models will not suffice. Using newer techniques for protecting the kidneys during prolonged surgery and interruption of blood flow based on successes in the field of transplantation and utilizing custom designed silastic catheters and fittings, some early success has been achieved in the creation of an awake intact dog model. It is anticipated that a successful model will be developed within the next few months which will enable the completion of this protocol. It is to be emphasized that the time spent on the creation of the dog model is necessary and important since it will represent an important advance in future renal studies. It is anticipated that this model will form the basis of future experimentation from this laboratory.

Calcium
Renin
Angiotensin
Hypertension
Dogs

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DMS/IN INSTR ^a	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
76 10 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	61102A	3A161102B71P	01	020			
B. CONTRIBUTING							
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) The Use of Lyophilized Venous Homografts for Peripheral AV Fistula in Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 12		76 09		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE: EXPIRATION:				PRECEDING		B. FUNDS (in thousands)	
B. NUMBER ^a				FISCAL YEAR		76 .5 14	
C. TYPE				CURRENCY			
D. KIND OF AWARD				E. CUM. AMT.			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research			
ADDRESS ^a Fort Sam Houston, Texas 78234				ADDRESS ^a Metabolic Branch Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME ^a Richard H. Merrill, LTC, MC			
TELEPHONE: 512-221-2720				TELEPHONE 512-221-5416			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: William D. Myers, LTC, MC			
				NAME: Thomas J. Lescher, MAJ, MC DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Homografts; (U) AV Fistula; (U) Vein graft; (U) Hyperalimentation; (U) Sepsis; (U) Thermal injury; (U) Humans							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Central venous catheters have long been implicated in contributing to systemic sepsis in hospitalized patients. This problem is especially critical in the thermally injured soldiers since infection is the principal cause of morbidity and mortality in this patient population. As the trauma patient is usually unstable and/or requires prolonged intravenous therapy for parenteral hyperalimentation and drug therapy, and any maneuver that would decrease the duration of intravenous catheter placement might well reduce the morbidity and mortality in these patients. Usually a central catheter is not required except for pressure monitoring and therefore a convenient peripheral access site would be desirable; however, in patients with massive burns peripheral sites are scarce and often short-lived. Creation of an artificial arterial-venous shunt in an extremity might well provide a more durable access which could be used not only for drug therapy and parenteral alimentation but also for recurrent diagnostic blood sampling.</p> <p>24. (U) Patients with burns ranging from 30-60% will be entered into this study. The patients will be assigned alternately to the study or control group. Those in the experimental group will have placed a peripheral lyophilized venous homograft within 48 hours of injury. A careful tabulation will be made in both groups of all complications of intravenous therapy. In the event of death all peripheral sites that have been cannulated will be inspected for evidence of infection or thrombosis, and evidence for systemic infection as a principal cause of death will be accessed by the pathologist.</p> <p>25. (U) 75 12 - 76 09 To date, two burn patients requiring prolong intravenous alimentation have had lyophilized cadaver allograft veins inserted as AV fistulae. In one patient graft sepsis necessitated removal two weeks post insertion. The other graft remained patent and free of sepsis and was ligated at the time of the patients discharge. Appropriate candidates are deemed to be rare even though the technic has been shown to be feasible.</p>							

TERMINATION

**PROJECT NO. 3A161102B71P-01 BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE**

**REPORT TITLE: THE USE OF LYOPHILIZED VENOUS HOMOGRAFTS FOR
PERIPHERAL AV FISTULA IN BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators

**Richard H. Merrill, MD, Lieutenant Colonel, MC
Hugh D. Peterson, DDS, MD, Colonel, MC
William D. Myers, MD, Lieutenant Colonel, MC
Thomas J. Lescher, MD, Major, MC**

Reports Control Symbol MEDDH-288 (RI)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3161102B71P-01 BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: THE USE OF LYOPHILIZED VENOUS HOMOGRAFTS FOR PERIPHERAL AV FISTULA IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

**Investigators: Richard H. Merrill, MD, Lieutenant Colonel, MC
Hugh D. Peterson, D.D.S., MD, Colonel, MC
William D. Myers, MD, Lieutenant Colonel, MC
Thomas J. Lescher, MD, Major, MC**

Reports Control Symbol MEDDH-288 (RI)

Central venous catheters have long been implicated in contributing to systemic sepsis in hospitalized patients. This problem is especially critical in the thermally injured patient since infection is the principal cause of morbidity and mortality in this patient population. It was for this reason that this investigation was initiated.

To date two burn patients requiring prolonged intravenous alimentation have had lyophilized cadaver allograft veins inserted as A-V fistula. In one patient graft sepsis necessitated removal 2 weeks post insertion. The other graft remained patent and free of sepsis and was ligated at the time of the patient's discharge. Appropriate candidates are deemed to be rare even though the technic has been shown to be feasible.

In the past eight months that the protocol has been in effect no further patients have been entered. It is the judgement of the primary physicians caring for the patients that suitable patients for this protocol will be few and therefore the study cannot be successfully concluded. As the input of patients into this study has been negligible, it is the feeling of the principal investigator that this study cannot be concluded within any reasonable time span utilizing this patient population, so the protocol is therefore terminated with no patients studied except for the two mentioned above.

Homografts
AV fistula
Vein graft
Hyperalimentation
Sepsis
Thermal Injury
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3 DATE PREV SUMMARY	4 KIND OF SUMMARY	5 SUMMARY SCTY ^a	6 WORK SECURITY ^a	7 REGRADING ^a	8A ORIGIN INSTR ^a	8B SPECIFIC DATA - CONTRACTOR ACCESS	9 LEVEL OF SUM
76 10 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10 NO / CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61102A		3A161102B71P		02 070	
b. CONTRIBUTING							
c. CONTRIBUTING							
11 TITLE (Proceed with Security Classification Code) (U) Determination of Arterial and Tissue Oxygen Tension in Normal Man to Develop a Technic to Measure Tissue Oxygen Tension in Burned Soldiers (44)							
12 SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13 START DATE		14 ESTIMATED COMPLETION DATE		15 FUNDING AGENCY		16. PERFORMANCE METHOD	
76 01		76 09		DA		C. In-House	
17 CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				b. PRECURSORS		b. FUNDS (in thousands)	
b. NUMBER:				FISCAL 76		.5	
c. TYPE:				YEAR CURRENCY		19	
d. KIND OF AWARD				f. CUM. AMT.			
19 RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: Clement L. Slade, CPT, MC			
TELEPHONE: 512-221-2720				TELEPHONE 512-221-4440			
21 GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Gary W. Welch, LTC, MC			
				NAME: Peter A. Petroff, MD DA			
22 KEYWORDS (Proceed EACH with Security Classification Code) (U) Oxygen; (U) Tension; (U) Arterial tissue; (U) Electrode; (U) Normal man							
23. TECHNICAL OBJECTIVE ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number proceed text of each with security Classification Code) 23. (U) To determine if a predictable relationship exists between arterial pO2 and tissue oxygen tension as measured by an indwelling oxygen electrode. If such a relationship exists in normal man then tissue oxygen tension may be a useful measure of peripheral perfusion in injured soldiers. 24. (U) Participants in this study will be members of the physician staff of the ISR. Detailed informed consent will be obtained. An oxygen electrode will be placed in the subcutaneous tissue in the muscle tissue of one arm and an indwelling arterial line will be placed in the brachial or radial artery of the other arm. Using the Ohio anesthesia apparatus F102 of 30 to 100% will be administered. Arterial pO2 and tissue pO2 will be simultaneously determined at each level of F102. 25. (U) 76 01 - 76 09 Five normal adults have been studied. In each subject there is a linear relationship between arterial pO2 and tissue oxygen tension. The slope of this line varies between subjects. There thus appears to be a range of normal. The shape of the curve suggests a relationship to age, physical conditioning and smoking history.							

^a Available to contractors upon originator's approval

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 65 AND 1498 1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

TERMINATION REPORT

PROJECT NO. 3A161102B71P-Q2, BASIC RESEARCH IN SUPPORT OF
MILITARY MEDICINE-SURGICAL PATHOLOGY

REPORT TITLE: DETERMINATION OF ARTERIAL AND TISSUE OXYGEN TENSION
IN NORMAL MAN TO DEVELOP A TECHNIC TO MEASURE TISSUE
OXYGEN TENSION IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

Clement L. Slade, MD, Captain, MC
Gary W. Welch, MD, Lieutenant Colonel, MC
Peter A. Petroff, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF
MILITARY MEDICINE-SURGICAL PATHOLOGY

REPORT TITLE: DETERMINATION OF ARTERIAL AND TISSUE OXYGEN TENSION
IN NORMAL MAN TO DEVELOP A TECHNIC TO MEASURE TISSUE
OXYGEN TENSION IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Clement L. Slade, MD, Captain, MC
Gary W. Welch, MD, Lieutenant Colonel, MC
Peter A. Petroff, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

It is now possible to measure tissue oxygen levels with an indwelling oxygen electrode which gives an instantaneous readout of tissue oxygen tension. It is likely that tissue oxygen tension is a function of blood flow in the area that is being evaluated. If such a relationship exists, tissue oxygen tension can be used as an index of tissue perfusion during resuscitation of thermally injured soldiers.

Tissue oxygen levels vary with blood oxygen levels (pO_2). Since pO_2 varies from patient to patient within a predictable range, it is important to know if a predictable, linear relationship exists between pO_2 and tissue oxygen. The useful data point in any study of peripheral perfusion would thus be the difference between pO_2 and tissue oxygen levels with a larger disparity expected when the area under investigation is receiving less blood flow.

The purpose of this study is to determine if a predictable relationship exists between pO_2 and tissue oxygen as measured by an indwelling oxygen electrode. Six normal human subjects were studied with a narrow gauge oxygen electrode (1.2 French, 0.16 [inch] placed percutaneously in the rectus femoris muscle. The electrode is attached to a light-weight meter (International Bio-Physics Cooperation). Simultaneous tissue oxygen and blood pO_2 measurements were made while inspired oxygen was varied between

room air and 100%. There was a wide range of pO_2 and muscle O_2 at high levels of inspired O_2 . At pO_2 of 100 to 200, a plot of pO_2 against muscle O_2 was linear and there was less variation from subject to subject. This is the range of pO_2 at which most trauma subjects will be studied. This system may thus prove to be a useful tool in the study of resuscitation of thermally injured soldiers.

Normal man
Electrode
Oxygen
Tension
Arterial tension

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
				DA OG 6962	76 10 01	DD-DRS(R)436	
3. DATE PREP SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DDD'S INSTR	9. SPECIFIC DATA - CONTRACTOR ACCESS	
76 10 01	H. TERM	U	U	NA	NL	<input type="checkbox"/> YES <input type="checkbox"/> NO A. WORK UNIT	
10. NO./CODES		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		61102A	3A161102B71P	01	022		
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) (U) Serum Pepsinogen Levels in the Thermally Injured Soldier: A Possible Predictor of Gastroduodenal Disease (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. FUNDING METHOD	
76 03		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES BY DATE			
Not Applicable				a. PERSONNEL (MAN YRS) b. FUNDS (\$ in thousands)			
a. DATES/EFFECTIVE:		EXPIRATION:		FISCAL YEAR		FUNDING YEAR	
b. NUMBER:		c. TYPE:		d. AMOUNT:		e. CUM. AMT.	
a. KIND OF AWARD:		b. PERCENTAGE OF AWARD:		c. PERCENTAGE OF AWARD:		d. PERCENTAGE OF AWARD:	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with N.E.S. Acronym if applicable)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: David K. Teegarden, MAJ, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2943			
21. GENERAL USE				22. ASSOCIATE INVESTIGATORS			
FOREIGN INTELLIGENCE NOT CONSIDERED				NAME: Dr. Samloff			
				NAME:			
23. KEYWORDS (Precede each with Security Classification Code) (U) Humans; (U) Stress ulcer; (U) Hemorrhagic gastritis; (U) Peptic ulcer disease following burn injury							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Precede individual paragraphs identified by number precede rest of each with Security Classification Code.)							
<p>23. (U) To determine if serum pepsinogen levels are a reliable predictor of gastroduodenal disease following burn injury in soldiers.</p> <p>24. (U) The method involved is sampling serum at regular intervals, and measuring the pepsinogen levels to determine trends and correlate this with clinical evidence of gastroduodenal disease in the burn patient.</p> <p>25. (U) 76 03 - 76 09 Eleven patients have been studied in pilot project. Although no definitive conclusion can be reached with such small numbers, two patients clearly showed elevation of the serum pepsinogen as analyzed by radioimmunoassay prior to gastrointestinal hemorrhage. The second phase of this study will be to correlate the serum pepsinogen levels with endoscopic findings.</p>							

TERMINATION REPORT

PROJECT NO. 3A161102B71P-01, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: SERUM PEPSINOGEN LEVELS IN THE THERMALLY INJURED SOLDIER:
A POSSIBLE PREDICTOR OF GASTRODUODENAL DISEASE

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

David K. Teegarden, M.D., Major, MC
I. M. Samloff, M.D.
(Professor of Medicine, UCLA)

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-01, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: SERUM PEPSINOGEN LEVELS IN THE THERMALLY INJURED SOLDIER:
A POSSIBLE PREDICTOR OF GASTRODUODENAL DISEASE

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: David K. Teegarden, M.D., Major, MC
I. M. Samloff, M.D. (Professor of Medicine, UCLA)

Reports Control Symbol MEDDH-288(R1)

Little attention has been directed to pepsin as an etiologic factor in ulcerative disease of the stomach and duodenum following thermal injury. Pepsin is a proteolytic enzyme that is formed from the cleavage of its precursor, pepsinogen. Its cells of origin are the chief cell and mucous neck cell in the stomach. Pepsinogen is a proteolytic enzyme which attacks soluble native proteins and is therefore an endopeptidase. Previous studies in duodenal ulcer disease and other diseases of the intestine have shown pepsinogen to increase prior to a gastrointestinal hemorrhage.

This study proposes to analyze serum pepsinogen levels and evaluate as a predictor of gastrointestinal erosion and ulceration following thermal injury.

Thermal injury
Burns
Stress ulceration
Hemorrhagic gastritis
Pepsinogen

SERUM PEPSINOGEN LEVELS IN THE THERMALLY INJURED PATIENT: A POSSIBLE PREDICTION OF GASTRODUODENAL DISEASE

Little attention has been given pepsin as an etiologic factor in ulcerative disease of the stomach and duodenum. Pepsin, a proteolytic enzyme, attacks soluble native proteins within the molecule and is therefore an endopeptidase. It is present in its cells of origin, the chief cell and mucous neck cells, as a zymogen precursor called pepsinogen. Other unidentified cells also contain pepsinogens. A leakage of digestive enzymes into the blood stream is a common occurrence and can be used, for instance, in detecting diseases of the pancreas, utilizing the serum lipase and amylase. How pepsinogens gain access to the circulation is unknown; serum levels have been shown to reflect the secretory activity and morphologic status of mucosa and have been helpful in the diagnosis of pernicious anemia, duodenal ulcer, and gastric cancer.

Pepsinogens are separated by immunochemical methods into two groups: PGI and PGII. The two groups differ in mucosal distribution and cellular origins. Both groups are found in the fundic mucosa.

The purpose of this study is to analyze the predictive value of the stomach enzyme precursor, pepsinogen, as an indicator of gastrointestinal hemorrhage resulting from stress erosions and ulceration following thermal injury.

Results

Eleven patients with a greater than 30% total body surface burn were included in a pilot study. The serum pepsinogen levels were measured daily for seven days, then every other day for the next seven days, and, finally, at weekly intervals for the next six weeks. The samples were submitted to Dr. Samloff's laboratory for analysis, utilizing a competitive binding double antibody radioimmunoassay.

Of the 11 patients in the pilot study, two patients demonstrated abnormally elevated serum pepsinogen levels, one of which was associated with a gastrointestinal hemorrhage. The remaining patients demonstrated essentially normal levels.

No specific conclusions can be drawn as to the reliability of the serum pepsinogen in reflecting morphology of the gastric mucosa; however studies correlating the serum pepsinogen level and endoscopic appearance of the stomach and duodenum will be performed in the future.

PRESENTATIONS AND PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY 76 10 01	4. KIND OF SUMMARY H. TERM	5. SUMMARY SCTY ^a U	6. WORK SECURITY ^a U	7. REGRADING ^a NA	8. DES'N INST'N NL	9. SPECIFIC DATA- CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	10. LEVEL OF SUM A. WORK UNIT
10. NO./CODES: ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61102A		3A161102B71P		02 067	
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Alterations in the Bacterial Flora of the Stomach and Upper Small Intestine Following Thermal Injury in a Military Population (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE 76 03		14. ESTIMATED COMPLETION DATE 76 09		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:		EXPIRATION:		FISCAL YEAR		b. FUNDS (in thousands)	
b. NUMBER ^a				76 7T		.3 .1	
c. TYPE:		d. AMOUNT:		TECHNICAL		7 1	
e. KIND OF AWARD:		f. CUM. AMT.					
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research			
ADDRESS ^a Fort Sam Houston, Texas 78234				ADDRESS ^a Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Pursue DDAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME ^a David K. Teegarden, MAJ, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2943			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: William S. McDougal, MAJ, MC			
				NAME: Truman M. Sasaki, MAJ, MC DA			
23. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Bacterial flora; (U) Intestinal flora; (U) Small bowel; (U) Humans							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Pursue individual paragraphs identified by number. Precede text of each with Security Classification Code)							
23. (U) To determine the nature of the bacterial flora of the stomach and small intestine following burn injury in soldiers.							
24. (U) A long intestinal tube will be inserted through the nose and bacterial samples will be obtained from the stomach and small bowel and submitted for culture technique.							
25. (U) 76 03 - 76 09 Hypomotility of the intestinal tract following thermal injury has limited the technique of gastrointestinal intubation. Until this technique has been further improved, cultures of the small bowel content cannot be obtained with reliability.							

^a Available to contractors upon contractor's approval

TERMINATION REPORT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: ALTERATIONS IN THE BACTERIAL FLORA OF THE STOMACH
AND UPPER SMALL INTESTINE FOLLOWING THERMAL INJURY
IN A MILITARY POPULATION

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

David K. Teegarden, M.D., Major, MC
William S. McDougal, M.D., Major, MC
Truman M. Sasaki, M.D., Major, MC
Barry L. Davison, M.S., Captain, MSC
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE, SURGICAL PATHOLOGY

REPORT TITLE: ALTERATIONS IN THE BACTERIAL FLORA OF THE STOMACH
AND UPPER SMALL INTESTINE FOLLOWING THERMAL INJURY
IN A MILITARY POPULATION

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: David K. Teegarden, M.D., Major, MC
William S. McDougal, M.D., Major, MC
Truman M. Sasaki, M.D., Major, MC
Barry L. Davison, M.S., Captain, MSC
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288(R1)

The role of the bacterial flora of the small intestine in infection of the thermally injured soldier has previously been undefined. Bacteria normally coexist in a relationship which is mutually beneficial to both the bacteria and the human. Under various conditions, these bacteria may proliferate with metabolic and infectious sequelae. Experimental studies have also shown that bacteria in the stomach may predispose to the development of stress ulceration.

This study proposes to investigate the bacterial flora of the stomach and small intestine in the thermally injured soldier.

Thermal injury
Infection
Stress ulceration
Bacterial flora

ALTERATIONS IN THE BACTERIAL FLORA OF THE STOMACH AND UPPER SMALL INTESTINE FOLLOWING THERMAL INJURY IN A MILITARY POPULATION

Bacteria are normal inhabitants in certain segments of the human intestine. They normally coexist in a relationship mutually beneficial to both organisms. Under normal situations, the stomach and upper small bowel are relatively sterile compared to the distal small bowel and colon, where luxuriant growth of bacteria is typical. Alterations in this balance between intestinal bacteria and man may result in adverse metabolic, biochemical, and infectious sequelae. It is well known that luxuriant bacterial growth in the small bowel may result in malabsorption, presumably by a deconjugation of bile acids, rendering intestinal juice with a bile acid concentration below the critical micellar concentration. This occurs primarily under conditions of impaired motility and stagnation of intraluminal contents from various causes. Intestinal organisms may also produce substances that either favor or inhibit the growth of other organisms. Transfer of antibiotic resistance from one organism to another is promoted by proliferation of various microbial strains. Bacteria also occupy a pivotal role in absorption of folate and Vitamin B-12.

The role of intestinal bacterial overgrowth in the pathogenesis of sepsis has not been established. Endotoxin of intestinal origin has been shown to be etiologically important in early deaths from large burns in animal studies. It can be postulated that hypoperfusion of splanchnic organs presumably occurs in the immediate postburn period. This might allow proliferation of organisms on an ischemic mucosa and possibly a port of entry into the circulation.

Following injury, patients with a total body surface burn of greater than 30% are being included in the study. A polyvinyl intestinal tube with sampling ports is employed to obtain samples from the stomach and upper small bowel near the ligament of Treitz. Samples of intestinal juice are collected within 72 hours of thermal injury and submitted for anaerobic and aerobic cultures.

Results

To date, luxuriant bacterial growth following thermal injury has not been established. A predominant organism has not appeared. A variety of both gram negative and gram positive organisms, as well as yeast, have been recovered. Although the colony concentrations are small, Klebsiella has been the predominant gram negative organism

recovered. Insufficient data has been collected to reach conclusions concerning the role of the bacterial flora of the stomach and small intestine following thermal injury.

PUBLICATIONS AND/OR PRESENTATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE (REV. SUMMARY)	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DMRN INSTR ^a	9. SPECIFIC DATA- CONTRACTOR ACCESS	10. LEVEL OF SUM A. WORK UNIT
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10. NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61102A		3A161102B71P		02	
b. CONTRIBUTING						068	
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Evaluation of Altered Metabolism of Phenylalanine and Tyrosine in Burned Soldiers (44).							
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13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 12		76 09		DA		C. In-House	
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a. DATES/EFFECTIVE:		EXPIRATION:		PRECEDING		b. FUNDS (in thousands)	
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c. TYPE:		d. AMOUNT:		FISCAL YEAR		3	
e. KIND OF AWARD:		f. CUM. AMT.		CURRENT			
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research			
ADDRESS ^a Fort Sam Houston, Texas 78234				ADDRESS ^a Burn Study Branch Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Platt, Jr, COL, MC				NAME ^a David N. Herndon, CPT, MC			
TELEPHONE: 512-221 2720				TELEPHONE: 512-221-4440			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: William S. McDougal, MAJ, MC			
				NAME: Douglas W. Wilmore, MD			
				DA			
23. KEYWORDS (Precede EACH with Security Classification Code) (U) Thyroid; (U) Phenylalanine; (U) Tyrosine; (U) Hypermetabolism; (U) Burns; (U) Catecholamines; (U) Humans							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) It is proposed to compare the kinetics of disappearance of oral phenylalanine and tyrosine loads in thermally injured septic and non-septic soldiers, versus normal controls.</p> <p>24. (U) Normal volunteers and septic and non-septic thermally injured patients with burns >40% of their total body surface (who have established normal GI function), will be asked to give informed consent and on successive days after 6-hour fasts be administered phenylalanine (.1g/kg/body wt) and tyrosine .05 g/kg body weight. Venous blood samples will be drawn at time 0 for basal concentrations of serum phenylalanine, tyrosine, glucose, and liver function tests. After the oral load of amino acids the blood will be drawn at hourly intervals for 6 hours and analyzed for tyrosine, phenylalanine and glucose. The amino acid levels will be measured in duplicate by specific fluorometric technics and on the amino acid analyser. Rate of disappearance of the 3 measured substances will be compared between normal, burned septic and burned non-septic patients.</p> <p>25. (U) 75 12 - 76 09 Phenylalanine and tyrosine administered as above to 11 thermally injured patients mean burn size 56% range 40-85% and 7 normals mean age 28. In burned patients fasting phenylalanine levels were markedly greater than normals 1.77±B 1.00=Norm. but rates of disappearance of phenylalanine and appearance of tyrosine after phenylalanine load were the same in normals as in burns. One hour glucose levels were however increased greater in burns. Δglucose burns = 22Δ glucose norm = 4. These data indicate that in burns peripheral breakdown of phenylalanine and its flow to glucose is greater than normal. Burns tyrosine tolerance tests were markedly lower than normals. They were comparable to tyrosine tolerance tests of myxedematous patients.</p>							

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TERMINATION

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE

REPORT TITLE: EVALUATION OF ALTERED METABOLISM OF PHENYLALANINE
AND TYROSINE IN BURNED PATIENTS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 December 1975 - 30 September 1976

Investigators:

David N. Herndon, MD, Cpt, MC
Douglas W. Wilmore, MD
Arthur D. Mason, Jr., MD
John Dubois, B.S.
Basil A. Pruitt, Jr, MD, Col, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY
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REPORT TITLE: EVALUATION OF ALTERED METABOLISM OF PHENYLALANINE
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US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 December 1975 - 30 September 1976.

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Previous studies at this institution have shown all serum amino acid levels with the sole exception of phenylalanine to be decreased in thermally injured patients. This study investigated abnormalities in phenylalanine and tyrosine metabolism in burned individuals from the 7th to the 14th post burn day. Seven controls were compared with 7 non-septic patients (total body surface burn 40 - 65%, mean 49%) and with 5 septic patients (total body surface burn 40-60%, mean 50%). Oral loads of 100 mg/kg of phenylalanine and 50 mg/kg tyrosine were administered to all subjects on separate days. Phenylalanine and tyrosine tolerance curves were determined from hourly post ingestion blood samples. The phenylalanine flow was at least equal to that in controls in the non-septic patients and was significantly ($p < 0.05$) increased in the septic patients indicating that an increase in phenylalanine level in thermally injured patients is an index of increased peripheral production and not due to decreased metabolism. Increases in serum tyrosine levels in response to an oral load were markedly lower in patients than in controls ($p < 0.01$) indicating a malabsorption of tyrosine similar to that observed previously in myxedematous patients. The rate of tyrosine disappearance following a phenylalanine load was increased in the non-septic patients relative to controls ($p < 0.05$).

Thyroid
Phenylalanine
Tyrosine
Hypermetabolism
Burns
Catecholamines

EVALUATION OF ALTERED METABOLISM OF PHENYLALANINE AND TYROSINE IN BURNED PATIENTS

Increased basal metabolic rate, protein catabolism, and weight loss characterize the metabolic response to major thermal injury. Recent evidence indicates that catecholamines mediate this hypermetabolic response.² Goodall and associates first demonstrated that increased urinary excretion of adrenaline and noradrenaline following large burns.³ The urinary excretion of catecholamines varies with the degree of injury and is related to the oxygen consumption of the patient.⁴ Catecholamine infusion in normal man increases metabolic rate,⁵ and combined alpha and beta adrenergic blockade or beta adrenergic blockade alone in a group of thermally injured patients significantly reduced metabolic rate.² Goodall and Haynes demonstrated that some patients with extensive thermal injuries have depleted adrenal medullary catecholamine stores.⁶ In additional studies, Goodall demonstrated an increase in conversion of dopamine to norepinephrine and suggested that dietary supplements of catechol precursors be added to the patients' diet to insure adequate catecholamine stores.⁷ A recent evaluation of serum amino acid levels in burn patients at this Institute⁸ demonstrated a reduction in the concentrations of all essential amino acids with the sole exception of phenylalanine which was elevated. Phenylalanine is a precursor in catecholamine synthesis and the phenylalanine to tyrosine ratio in normal man is approximately 1.⁹

1. Wilmore DW: Nutrition and metabolism following thermal injury. Clin Plast Surg 1: 603, 1975.

2. Wilmore DW, Long JM, Mason AD, Jr, Skreen RW, Pruitt BA, Jr: Catecholamines: mediator of the hypermetabolic response to thermal injury. Ann Surg 180: 653, 1974.

3. Goodall, McC, Stone C, Haynes BW, Jr: Urinary output of adrenaline and noradrenaline in severe thermal burns. Ann Surg 145: 479, 1957.

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5. Stone DJ, Katz H, Sarkor TK, Singzon J: Ventilatory response to alpha-adrenergic stimulation and inhibition. J Appl Physiol 34: 619, 1973.

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8. Coleman RA, Wilmore DW: Unpublished data, 1975.

9. Perry HL, Trachler B, Hansen S, MacDougal I. A simple test for heterozygosity for phenylketonuria. Clin Chim Acta 15: 47-55, 1962.

This ratio in burned patients is elevated to approximately 1.6.⁸ Similar elevations in intracellular phenylalanine levels have been observed in surgically stressed patients¹⁰ and in the serum levels of children with kwashiorkor and malnutrition.^{11,12} It is unknown if this elevation of phenylalanine is due to a block of its conversion in the liver to tyrosine or glucose, or whether it is secondary to increases in catabolism of protein in the periphery. This study was designed to determine whether flow of phenylalanine and tyrosine is abnormal in the thermally injured patient. This information may determine whether dietary supplementation with these catechol precursors or co-factors of their enzymes would increase the catecholamine stores thereby increasing reserves in stressed, thermally injured patients.

BACKGROUND

Phenylalanine and tyrosine metabolism have been extensively studied because in phenylketonuria there is a congenital absence of the enzyme phenylalanine hydroxylase which converts phenylalanine to tyrosine.¹³ Evaluation of parents of homozygotes for this recessive disease has demonstrated that conversion of phenylalanine to tyrosine is decreased in heterozygotes as well as homozygotes.¹⁴ Other studies¹⁴⁻¹⁹ have demonstrated that serum concentrations of phenylalanine following an oral load of phenyl-

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15. Hsia DYY, Paine RS: Phenylketonuria detection of the heterozygous carrier. *J Ment Defic Res* 1: 53, 1957.

16. Hsia DYY: Phenylketonuria the phenylalanine-tyrosine ratio in the detection of the heterozygous carrier. *J Ment Defic Res* 2: 8, 1958.

17. Hsia DY, Driscoll KW. Detection of the heterozygous carriers of phenylketonuria. *Lancet* 271: 1337 (29 Dec) 1956.

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alanine ranging from 0.1 to 0.33 gm/kg/body weight are increased in heterozygotes when compared to the curves generated in normal man. Several investigators^{16,18,20,21} have followed serum tyrosine levels in heterozygotes after phenylalanine loading and found it to be significantly lower than the tyrosine response generated from the same phenylalanine load in normal man. Evaluation of these individuals using intravenous loading technics showed no discriminating improvement with intravenous loading compared with oral loading tests⁹. Phenylalanine conversion to tyrosine and tyrosine tolerance tests are also abnormal in certain liver diseases.²² In all of these studies large oral loads of phenylalanine and tyrosine were given to a wide variety of patients. This study analyzes phenylalanine and tyrosine metabolism by similar technics in thermally injured patients.

MATERIALS AND METHODS

Seven control subjects, 4 males, 3 females, ages 23-34, mean age 28, all with no past history of liver disease, no family history of phenylketonuria and with normal liver function tests at the time of the study were compared with 5 septic burn patients (defined by having positive blood cultures on the day of the study, 2 patients had Klebsiella sepsis and 3 had Pseudomonas sepsis). Their burn sizes ranged from 40-65% of total body surface, mean 50% total body surface. Their ages varied from 19-60, with a mean age of 49. Controls and septic burns were compared with 7 uncomplicated, thermally injured patients, burn size 40-65% of total body surface, mean 49% of total body surface. Their ages varied from 19-50 years of age with a mean of 31. The burned patients were studied on the 7th to 14th post burn days.

After a 12-hour fast, patients and controls were given (1) an oral load of phenylalanine of 100 mg/kg at time zero. The phenylalanine was made palatable by mixing with 100 gm of dietetic applesauce. Blood was drawn at hourly intervals starting prior to ingestion and running to 5 hours post-ingestion. Serum was analyzed by colorimetric methods (Sigma kit) for phenylalanine and tyrosine levels, and for glucose levels. (2) At a time 3 to 5 days after the phenylalanine loading tests, patients and controls were given 50 mg/kg of tyrosine orally, made palatable by mixing with applesauce. Blood was drawn at hourly intervals starting prior to ingestion and running to 5 hours post-ingestion. Serum was analyzed for phenylalanine, tyrosine and glucose levels as above. Values at fasting and each hourly interval post-oral load were compared between the three groups by analysis of variance using Scheffe technic for multiple comparisons. When discrete disappearance curves could be defined by linear regressions, these were compared by analysis of variance and their rates of disappearance determined from the regression slope constants.

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RESULTS

A. Phenylalanine Tolerance Tests, 100 mg/kg Administered Orally at Time Zero. When the appearance-disappearance curves of phenylalanine in mg/100 cc of serum versus time in response to an oral load of 100 mg/kg of phenylalanine of the 7 controls are compared with all 12 burn patients (Fig. 1), the fasting levels are not statistically separate nor are the 4 and 5 hour post-ingestion values. The 1, 2, and 3 hour post-ingestion values for the patients are significantly decreased below those of the controls ($p < 0.025$, $p < 0.01$, and $p < 0.001$ respectively). A regression line fit from the 1 to 4 hour, mean values for the controls has an equation $Y = 14.7921 - 2.3893X$ with an r^2 of 0.9808, a similar line for the patients has an equation $Y = 10.0729 - 1.6304X$ with an r^2 of 0.9075. By analysis of variance, the difference in the slopes of these lines is not statistically significant but the control line is relatively elevated over that of the patients, $p < 0.01$.

When the appearance-disappearance curves for tyrosine in mg/100 cc of serum versus time in hours in response to a 100 mg/kg phenylalanine oral load were plotted, (Fig 2) the responses of the patients could not be statistically separated from the responses of the controls.

The glucose levels in response to the above load were significantly higher in all of the patients relative to controls at all times, $p < 0.01$ (Fig. 3); but there was no change in response to the phenylalanine load from fasting levels in either group (Fig. 4).

When the 5 septic patients are compared to the 7 controls, the phenylalanine tolerance curve in mg/100 cc/hour shows the septic patients fasting levels to be significantly greater than that of the controls. (Septic patients mean mg phenylalanine/100 cc serum at time Zero = 2.330 with a standard deviation of 0.74; controls mean mg phenylalanine/100 cc serum at time Zero = 1.1514 with a standard deviation of 0.38; $p < 0.01$ (Fig. 5). Since the fasting levels are different in these 2 groups, disappearance rates must be calculated from changes from fasting levels (Fig. 6). The equation defining the disappearance of the controls is $Y = 2.39X + 13.64$, $r^2 = 0.98$. That for the septic patients is $Y = 2.03X + 10.3938$, $r^2 = 0.92$. The slopes of these lines, or the rates of disappearance, are statistically equal. The increases from fasting are statistically equal at 1 and 2 hours post-ingestion but at 3, 4, and 5 hours post-ingestion the increases are significantly greater in the controls than in the patients ($p < 0.01$). The tyrosine appearance-disappearance curves to the phenylalanine loads were the same in the controls and in the septic burn patients (Fig. 7).

When the 7 non-septic or uncomplicated burns are compared to the 7 controls, the fasting phenylalanine levels and rates of appearance and disappearance of phenylalanine are identical, but the patient curve is significantly lower at all times beyond fasting, $p < 0.001$ (Fig. 8). The tyrosine appearance-disappearance curves are notable in that the fasting tyrosine level is greater in the uncomplicated burn patients than in the controls (Fig. 9). Therefore, appearance-disappearance rates in this instance must be

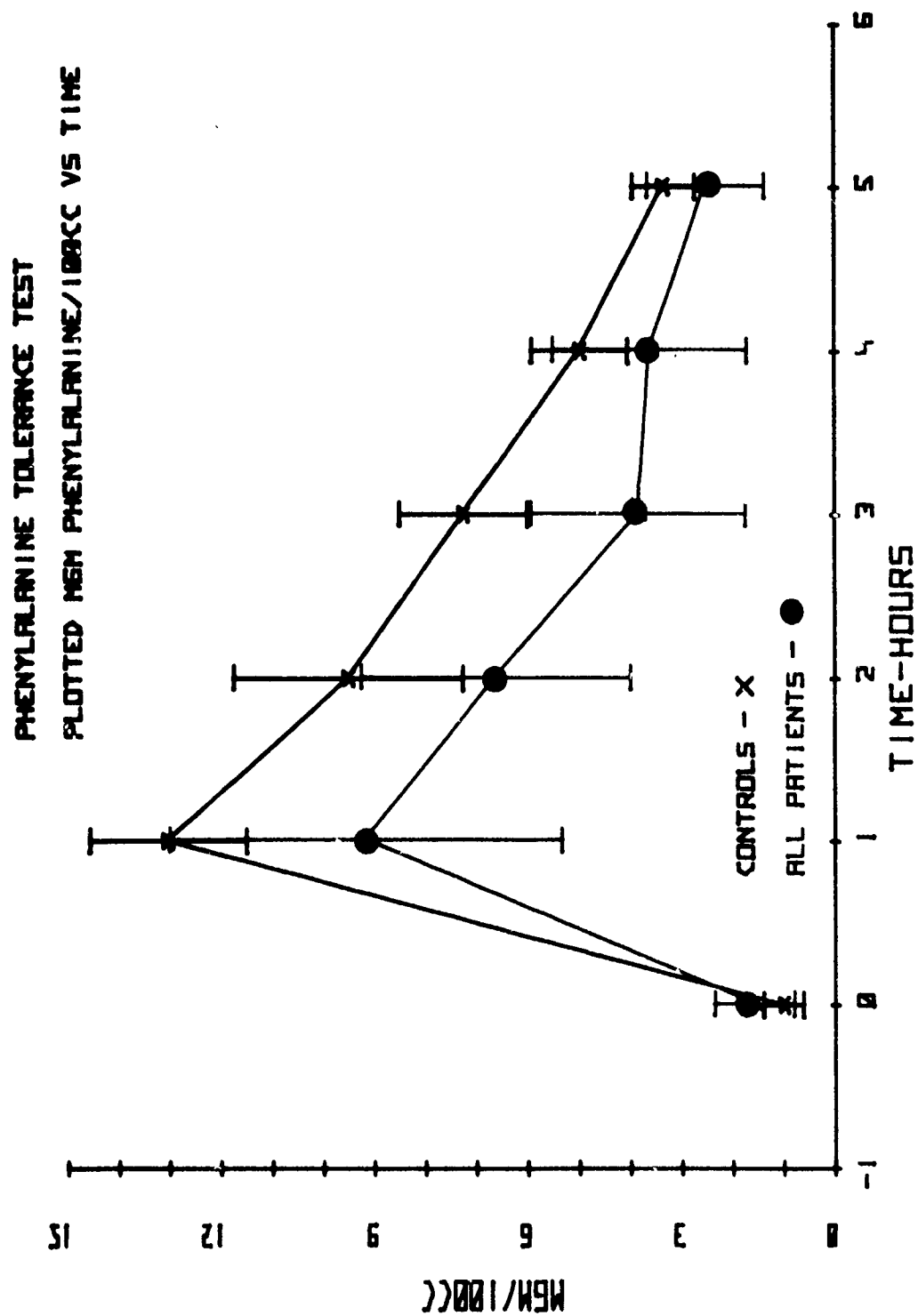


Figure 1

PHENYLALANINE TOLERANCE TEST PLOTTED: MG% TYROSINE/100CC VS TIME

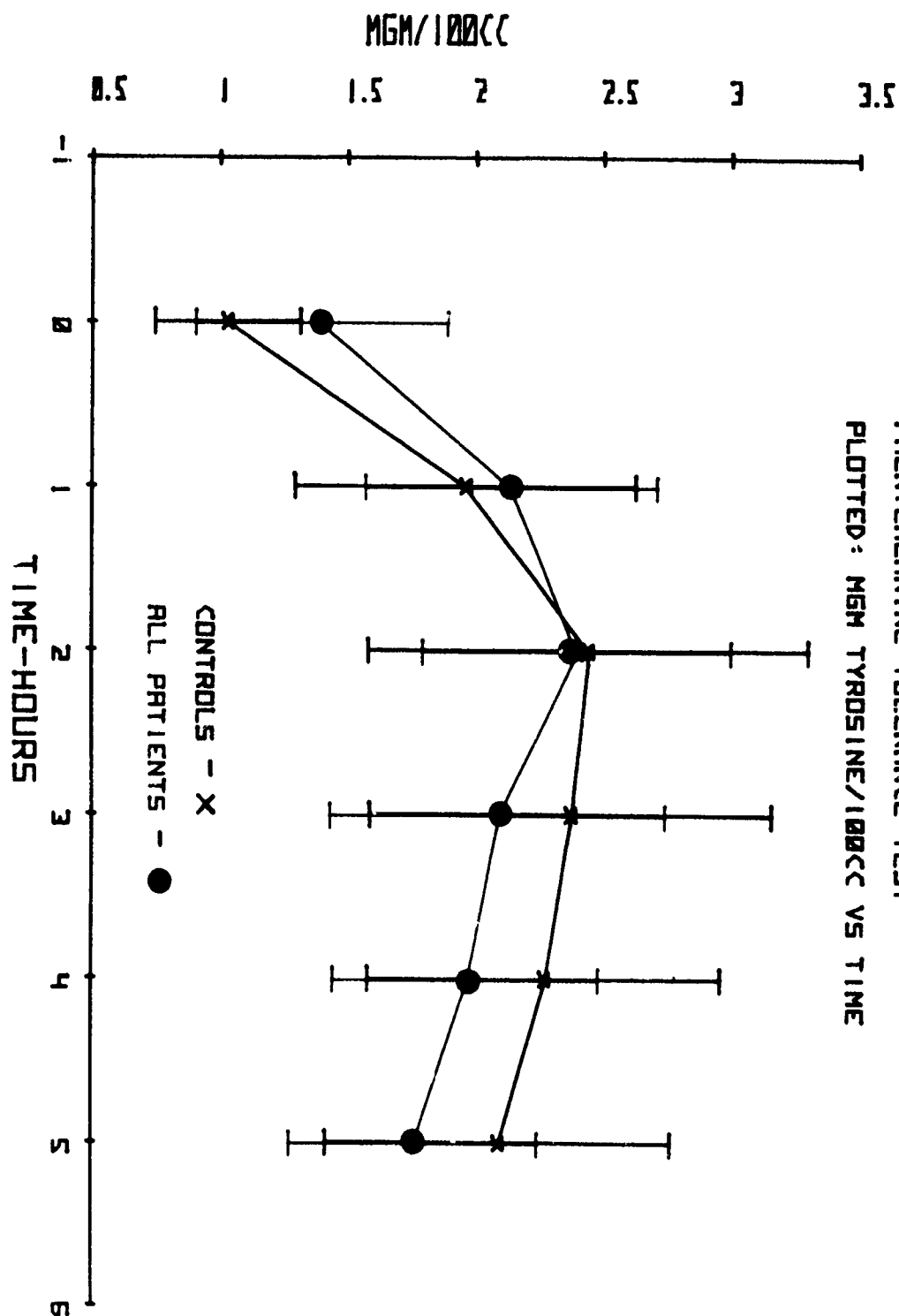


Figure 2

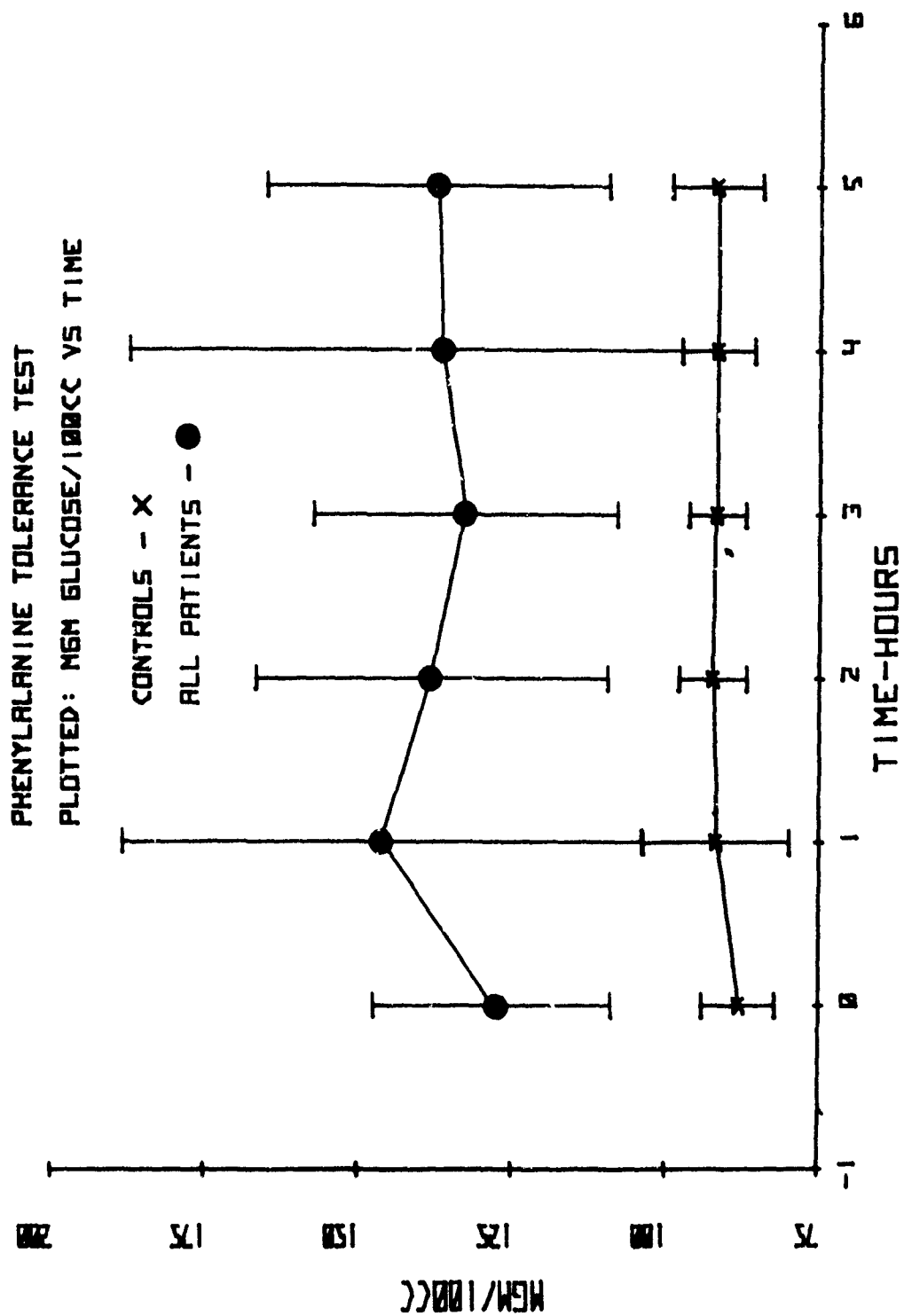


Figure 3

PHENYLALANINE TOLERANCE TEST
 PLOTTED: DELTA MG% GLUCOSE/100CC VS TIME

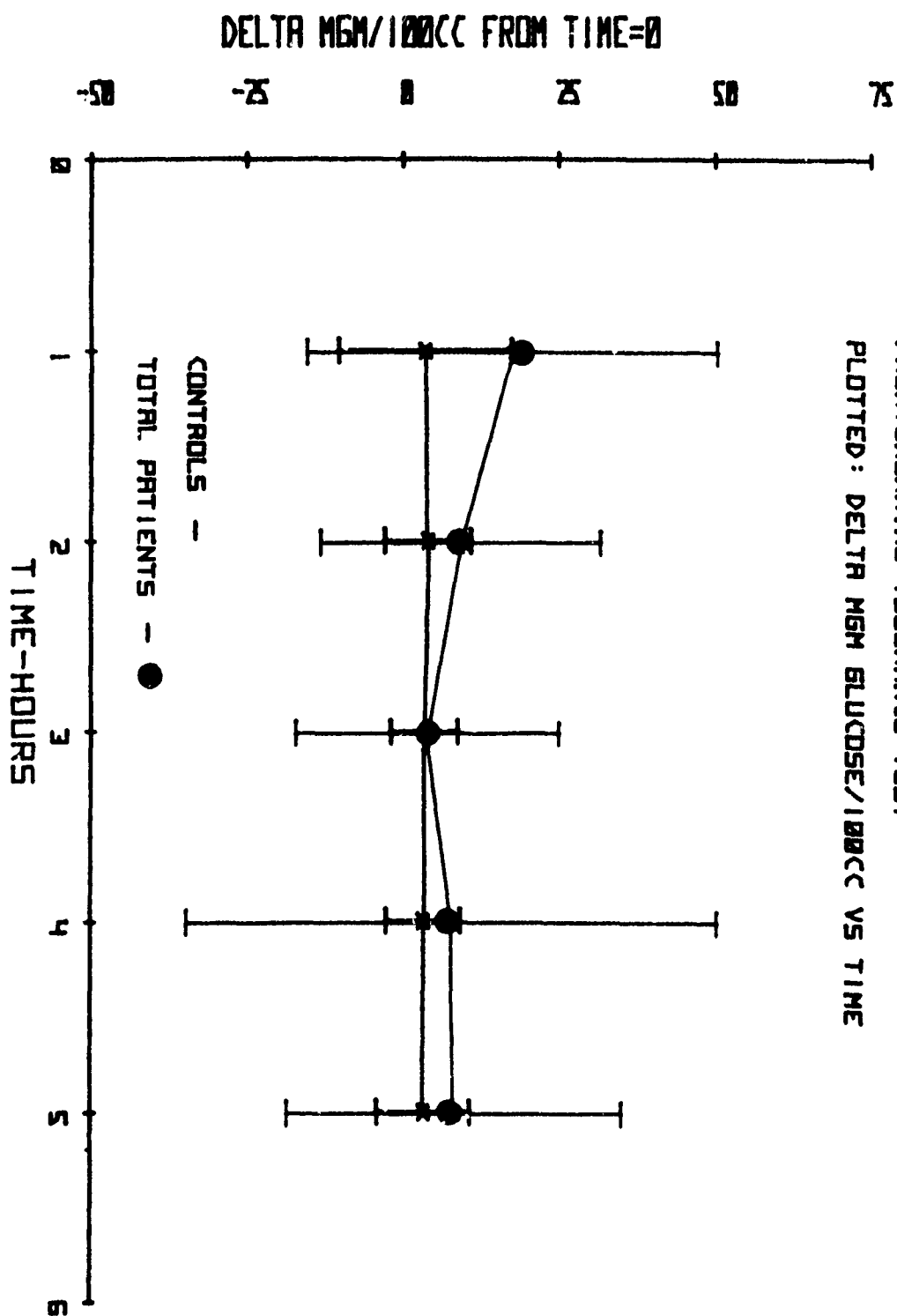


Figure 4

PHENYLALANINE TOLERANCE TEST
 PLOTTED: MSN/100CC VS TIME

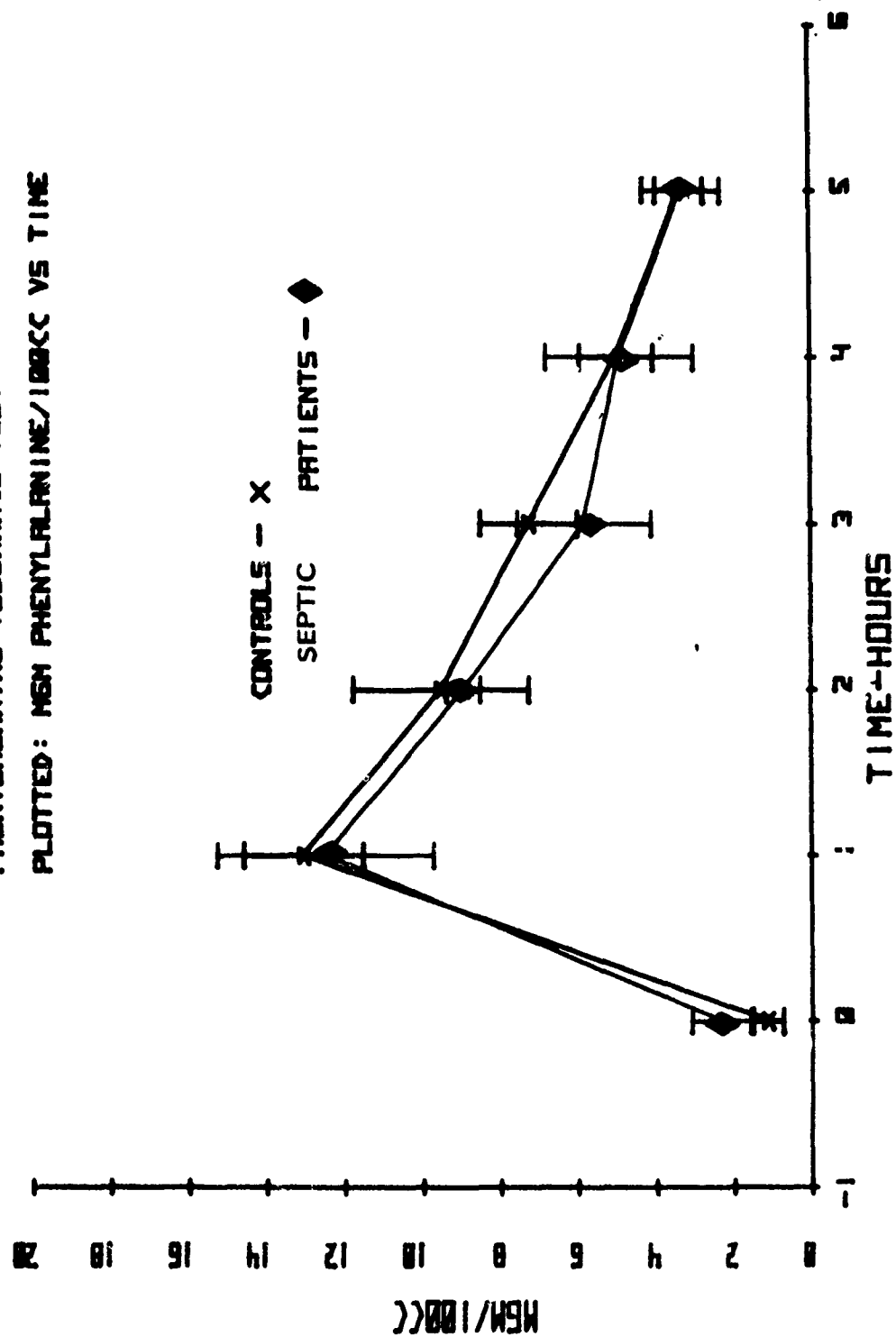


Figure 5

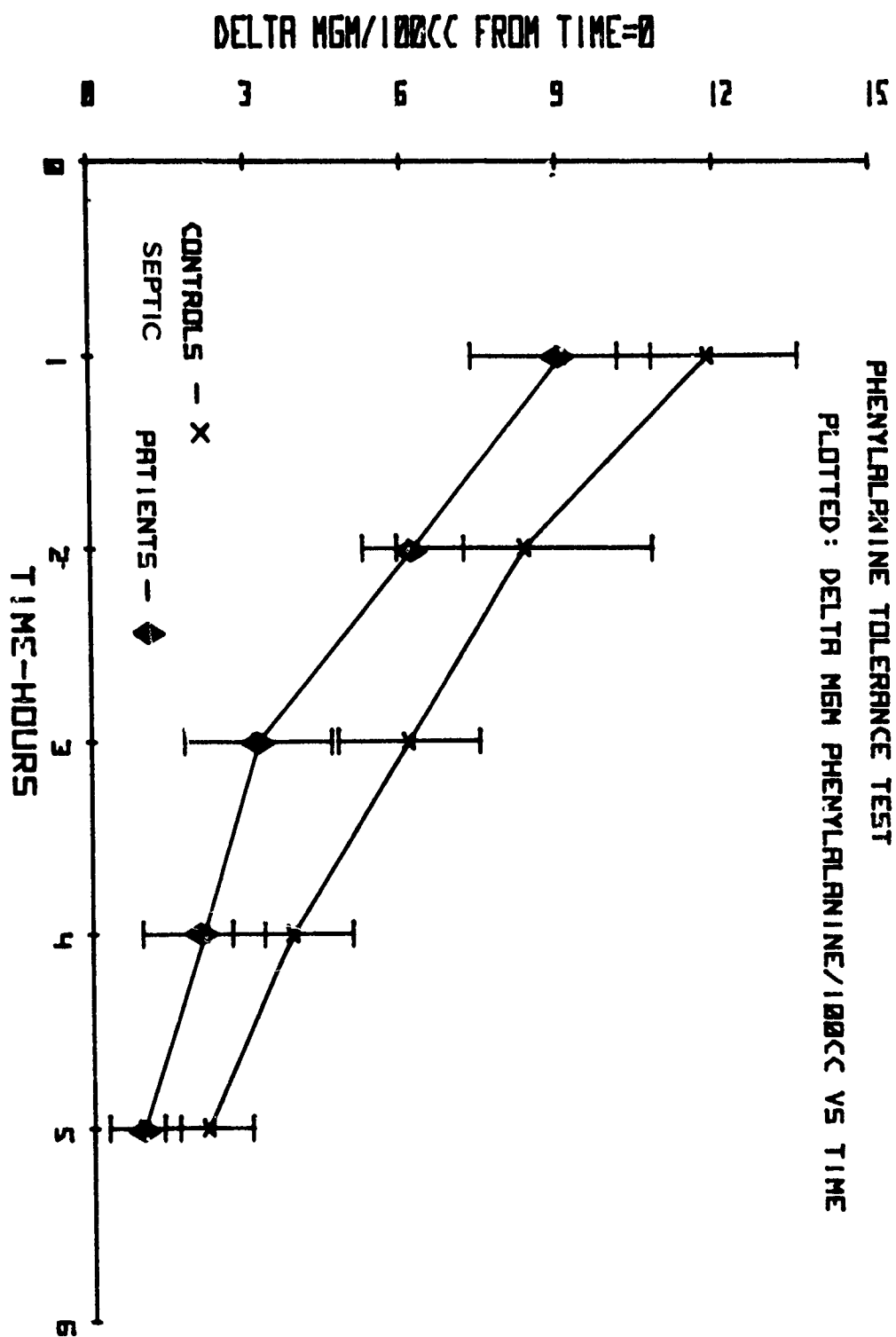


Figure 6

PHENYLALANINE TOLERANCE TEST
PLOTTED: MG% TYROSINE / 100CC VS TIME

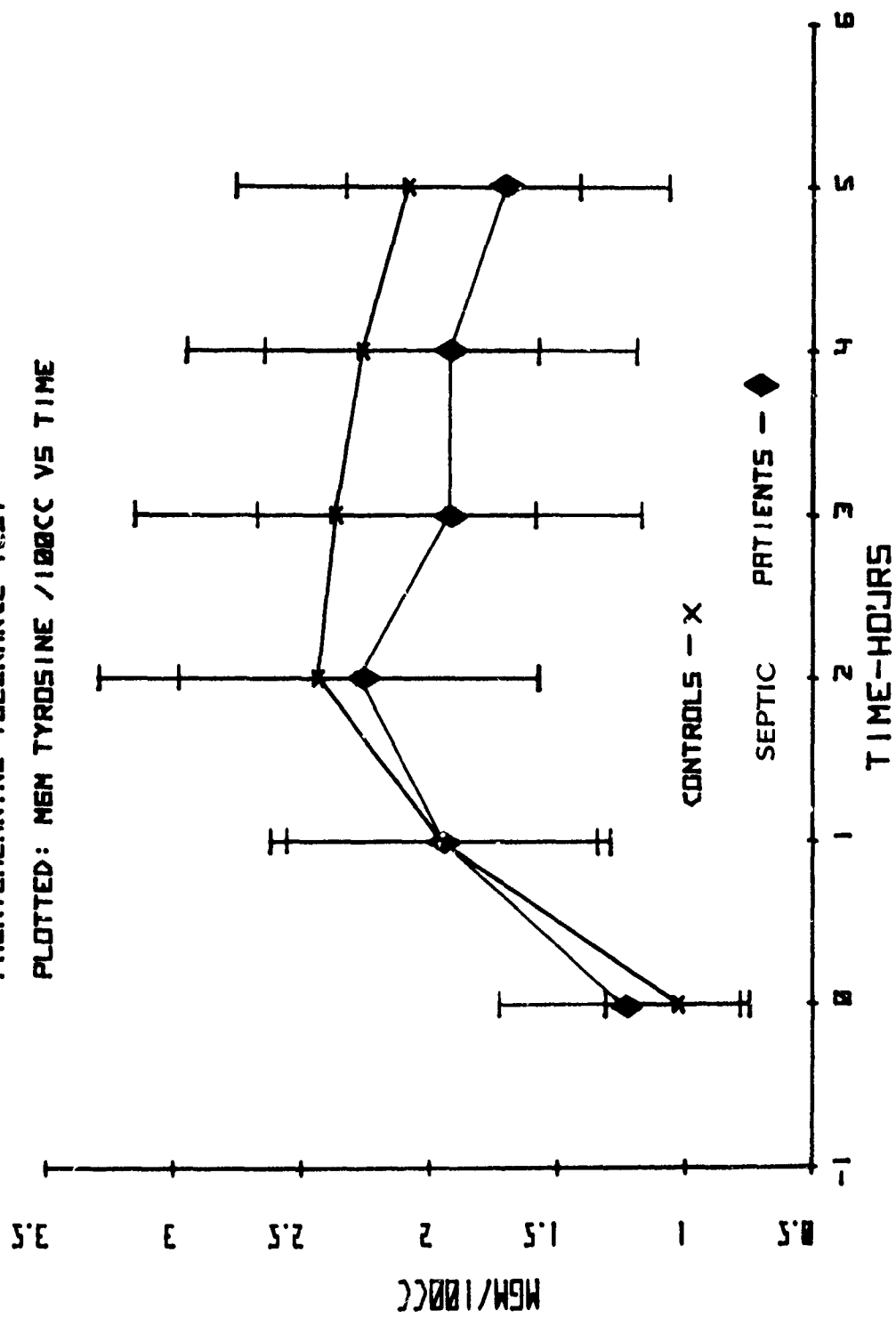


Figure 7

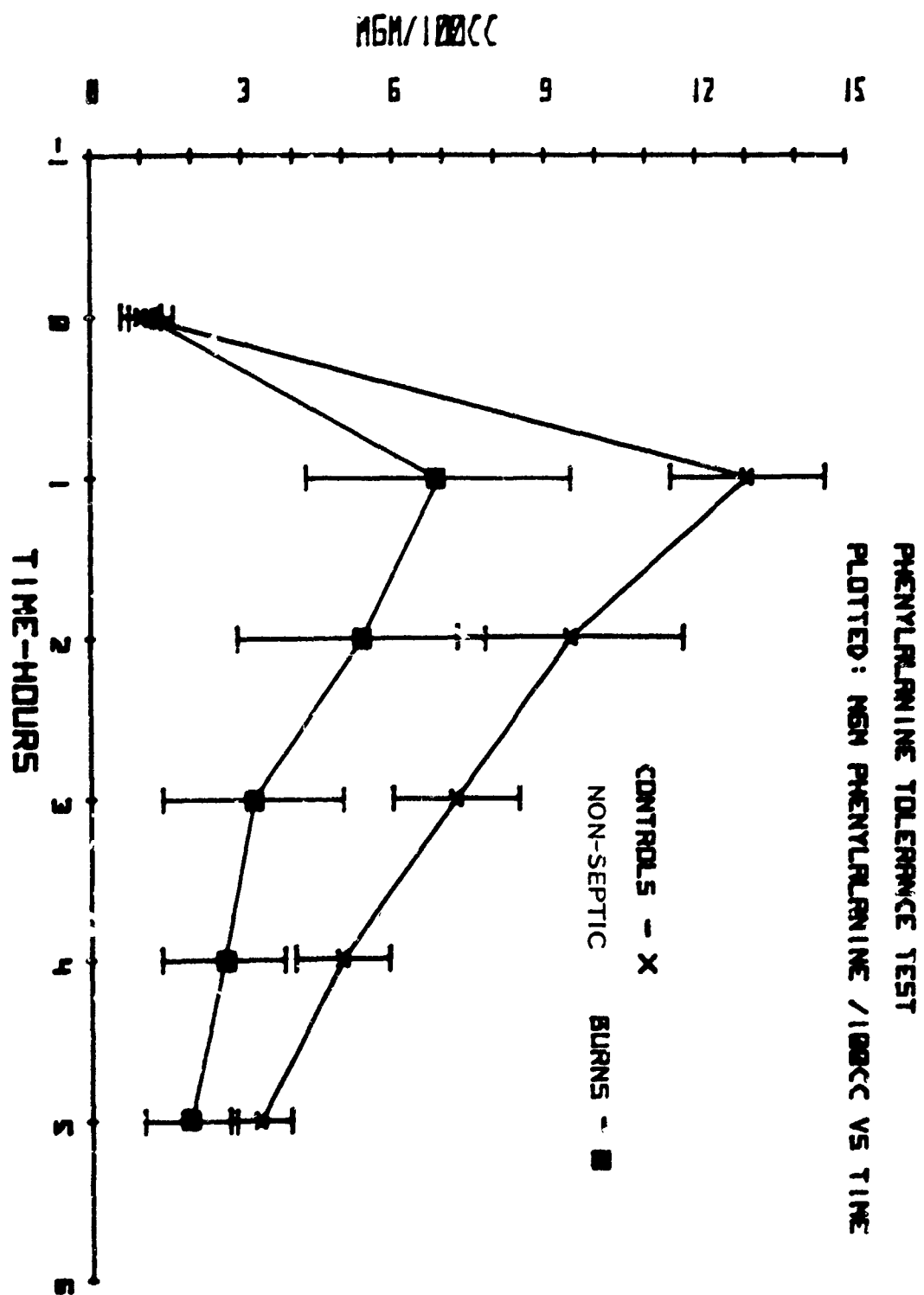


Figure 8

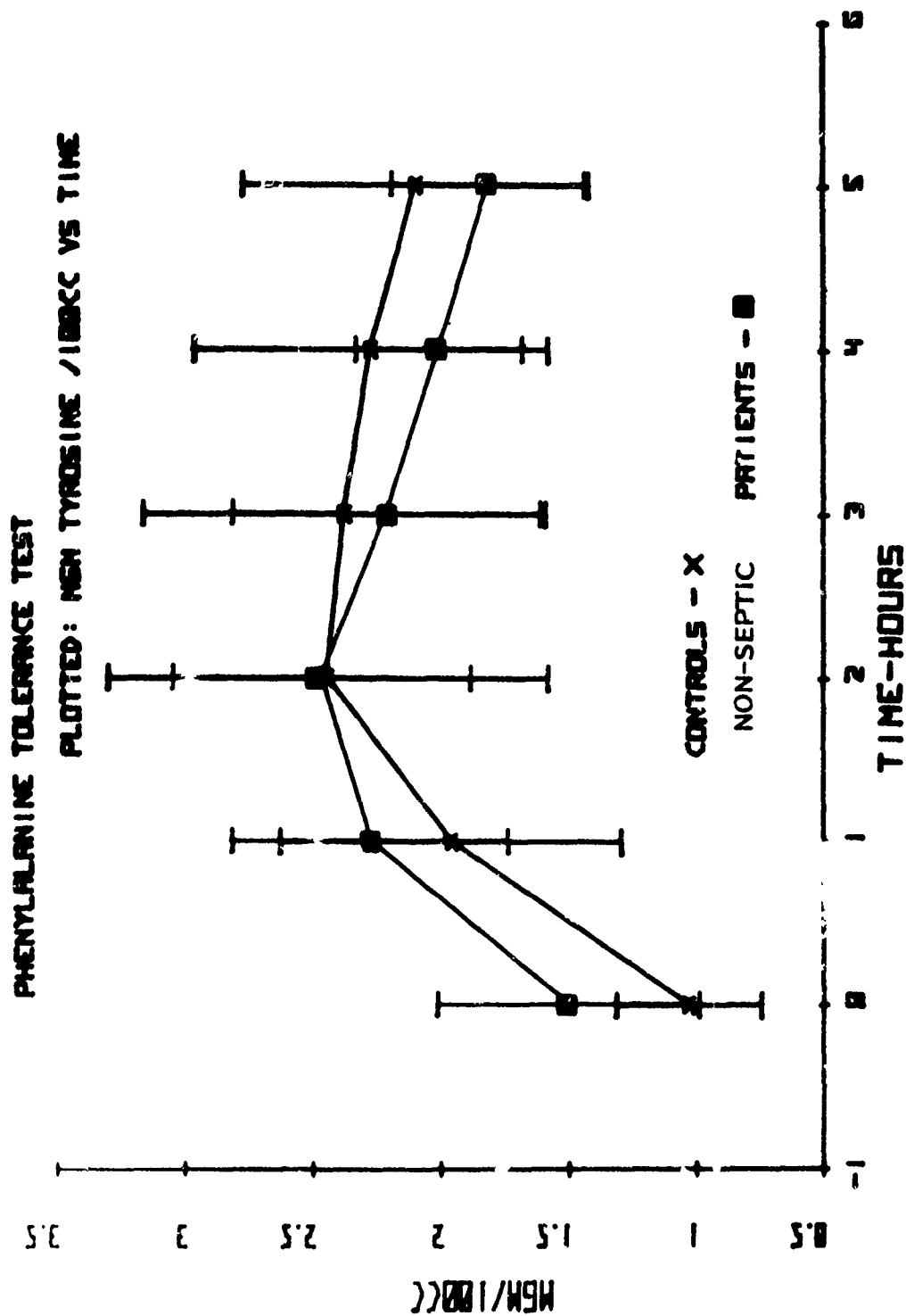


Figure 9

examined by comparing changes from fasting as in Figure 10. When this is done, there is no difference in the 2, 3 and 5 hour changes from fasting between the 2 groups, but the 4 hour values are significantly lower in the uncomplicated burn group than in the control group ($p < 0.05$). The regression line describing control disappearance from 2 to 4 hours post-ingestion of phenylalanine is described by the equation $Y = 0.1150X + 1.6628$ with an r^2 of 0.96; that for the uncomplicated patients is $Y = 0.2427 + 1.4299$ with an r^2 of 0.98. The slope, or rate of disappearance for the burns is significantly greater in the non-septic burned group than in the control group, $p < 0.05$.

If the changes from fasting phenylalanine levels in response to 100 mg/kg oral dose of phenylalanine of the septic and non-septic patients are compared as in Figure 11, the regression line describing the uncomplicated controls is $Y = 6.387 - 1.2899X$ with an r^2 of 0.94 and the line for the septic burns is $Y = 10.3938 - 2.0318X$ with an r^2 of 0.95. The septic patients have a greater elevation at one hour $p < 0.05$, and a greater slope from 1 - 5 hours, $p < 0.05$.

B. Serum Tyrosine Response in mg/100 cc of Serum to 50 mg/kg Tyrosine Oral Load at Time Zero. When changes in levels of phenylalanine and glucose from fasting are compared after a 50 mg/kg tyrosine oral load no statistical difference at any period could be discerned between any group of patients and controls (Fig. 12, 13). When the tyrosine appearance-disappearance curves are compared for this tyrosine load, the rates of disappearance could not be differentiated but the degree of elevation from fasting was only a fraction in the patients of that seen in the controls at any given time post-ingestion, $p < 0.01$ (Fig. 14). The curves of septic and non-septic patients could not be distinguished.

DISCUSSION

A recent review of the serum concentrations of all essential amino acids in the thermally injured patients treated at this Institution showed all essential amino acids to be decreased with the sole exception of phenylalanine.⁸ Examination of the major pathway for phenylalanine metabolism (Fig. 15) would suggest that increased phenylalanine levels could be from either increased production of phenylalanine, decreased conversion of phenylalanine to tyrosine, or shunting of phenylalanine to other metabolic pathways such as gluconeogenesis. Our data show that the rate of disappearance of phenylalanine, and its conversion to tyrosine are equal in uncomplicated and complicated burns and in normals. In septic burns, since their fasting phenylalanine levels are greater than controls, the phenylalanine flow which is equal to the fasting concentration times the rate of disappearance, is increased. It is probable that the phenylalanine flow is even greater than suggested by these data as the extracellular water of thermally injured patients from the 7th to the 14th post burn day is greater than that of controls. This would cause the space occupied by phenylalanine to be larger in patients than in controls. Phenylalanine flow would be increased by the proportion by which this space is enlarged. Elevated phenylalanine levels in burned patients are then secondary to increased peripheral production and not due to decreased conversion to tyrosine. Phenylalanine levels can be taken to represent a

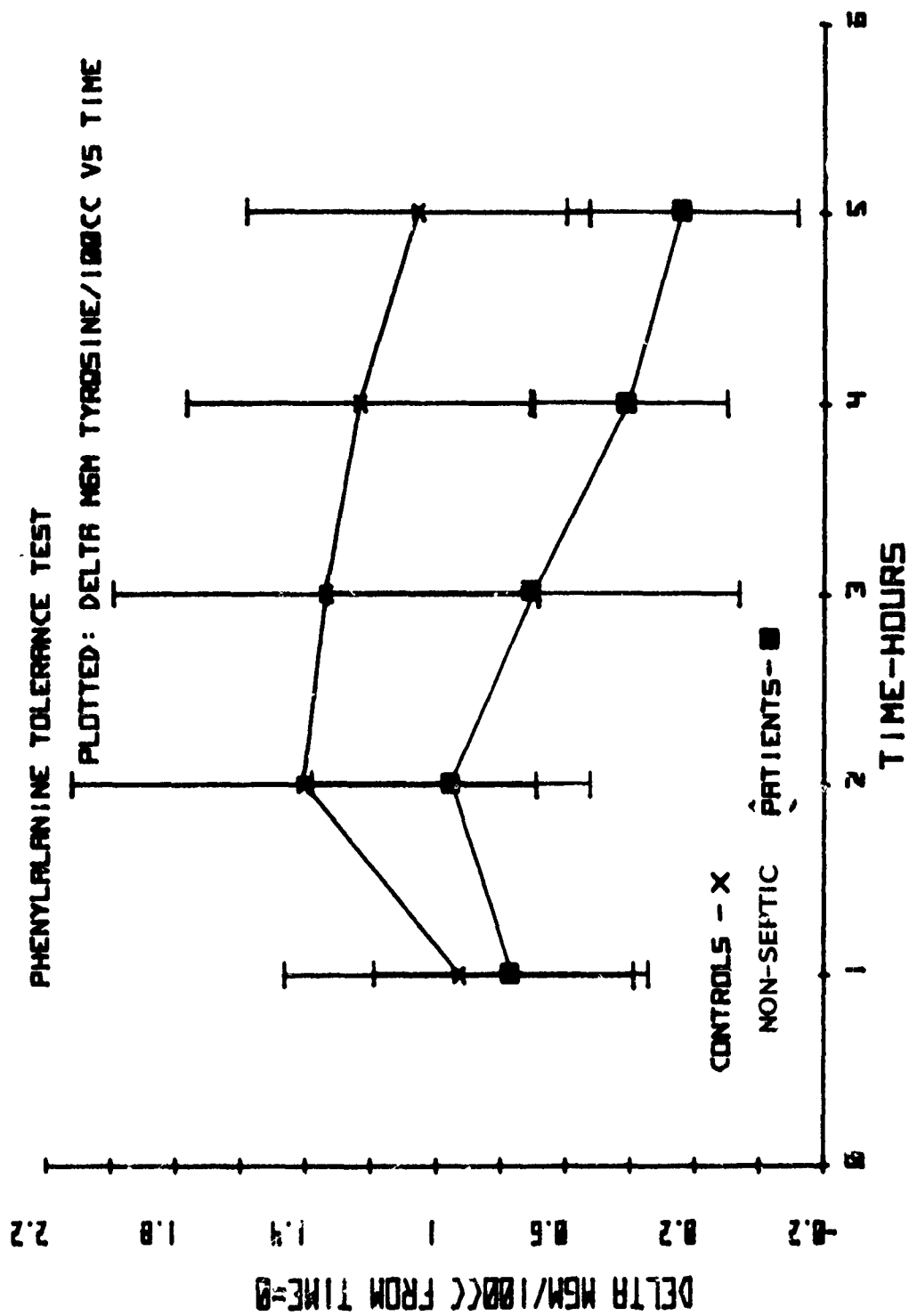


Figure 10

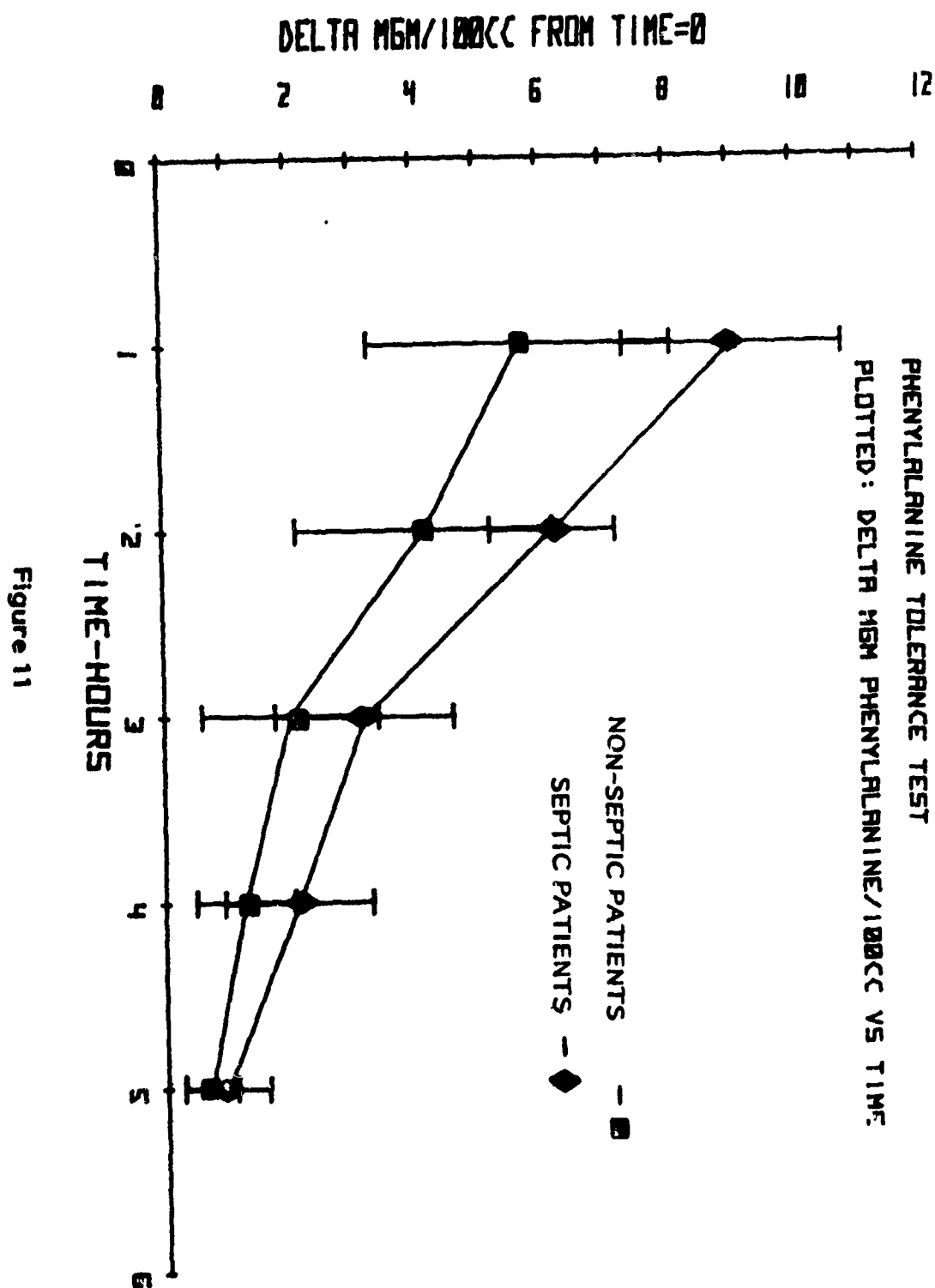


Figure 11

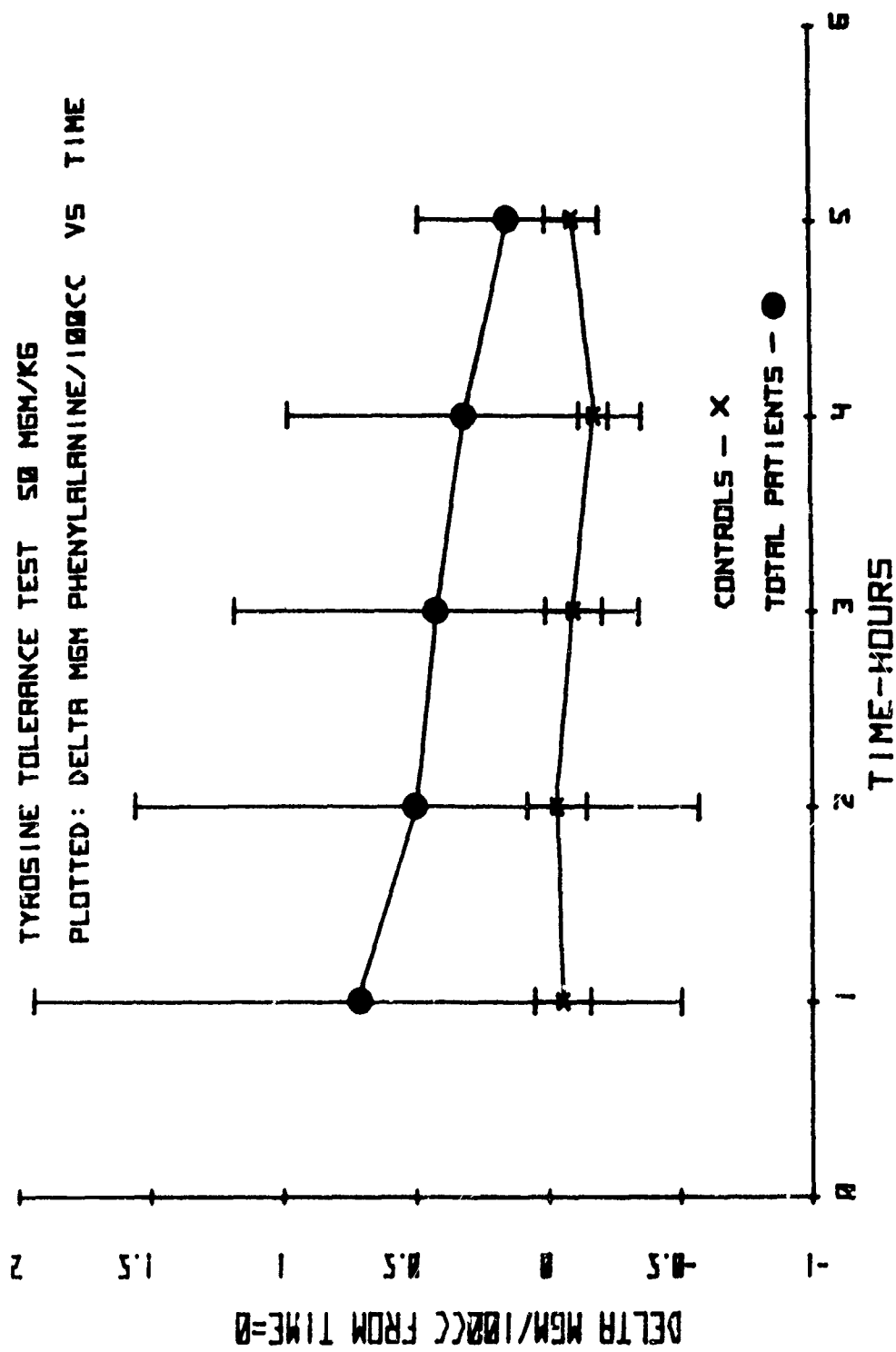


Figure 12

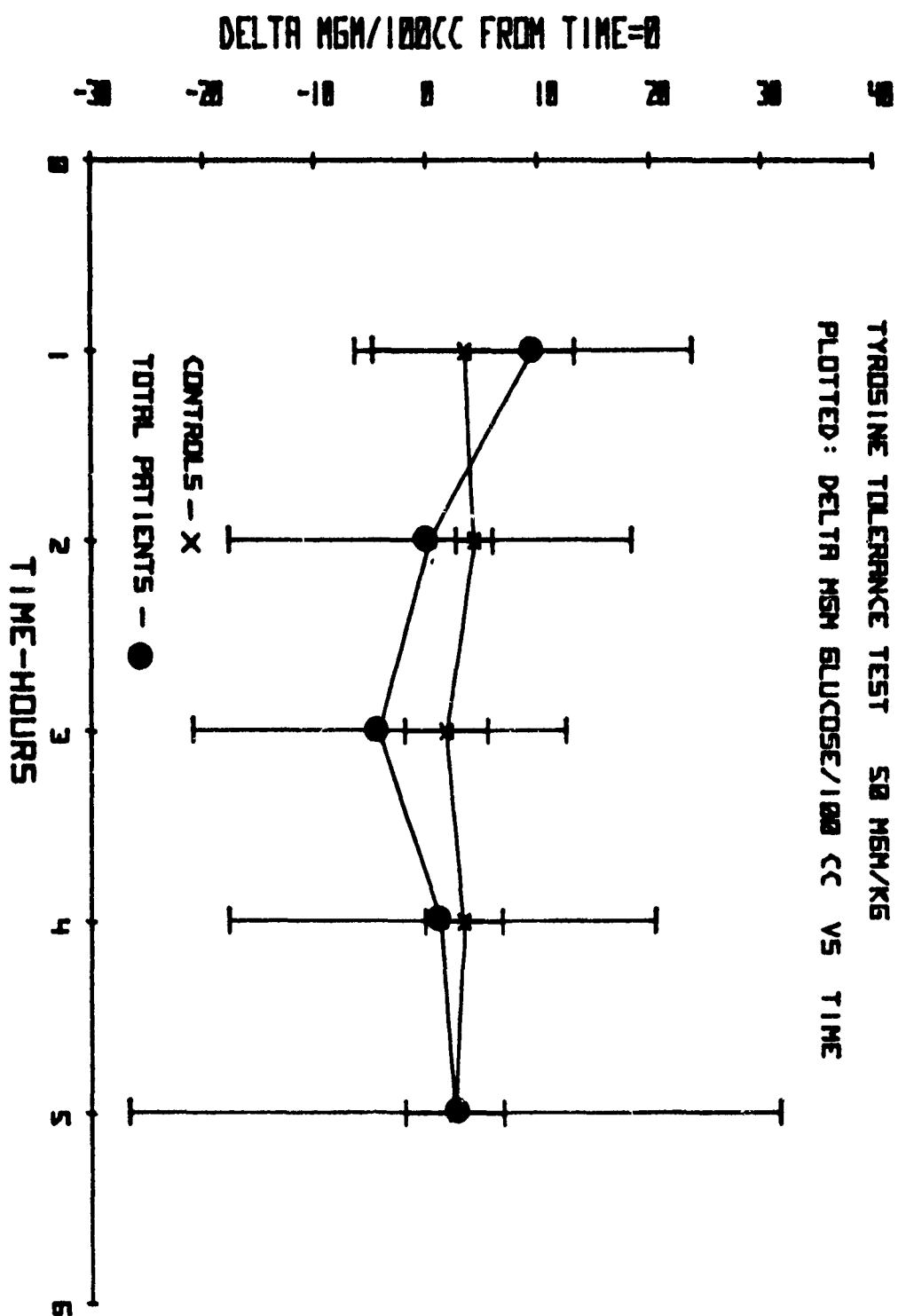


Figure 13

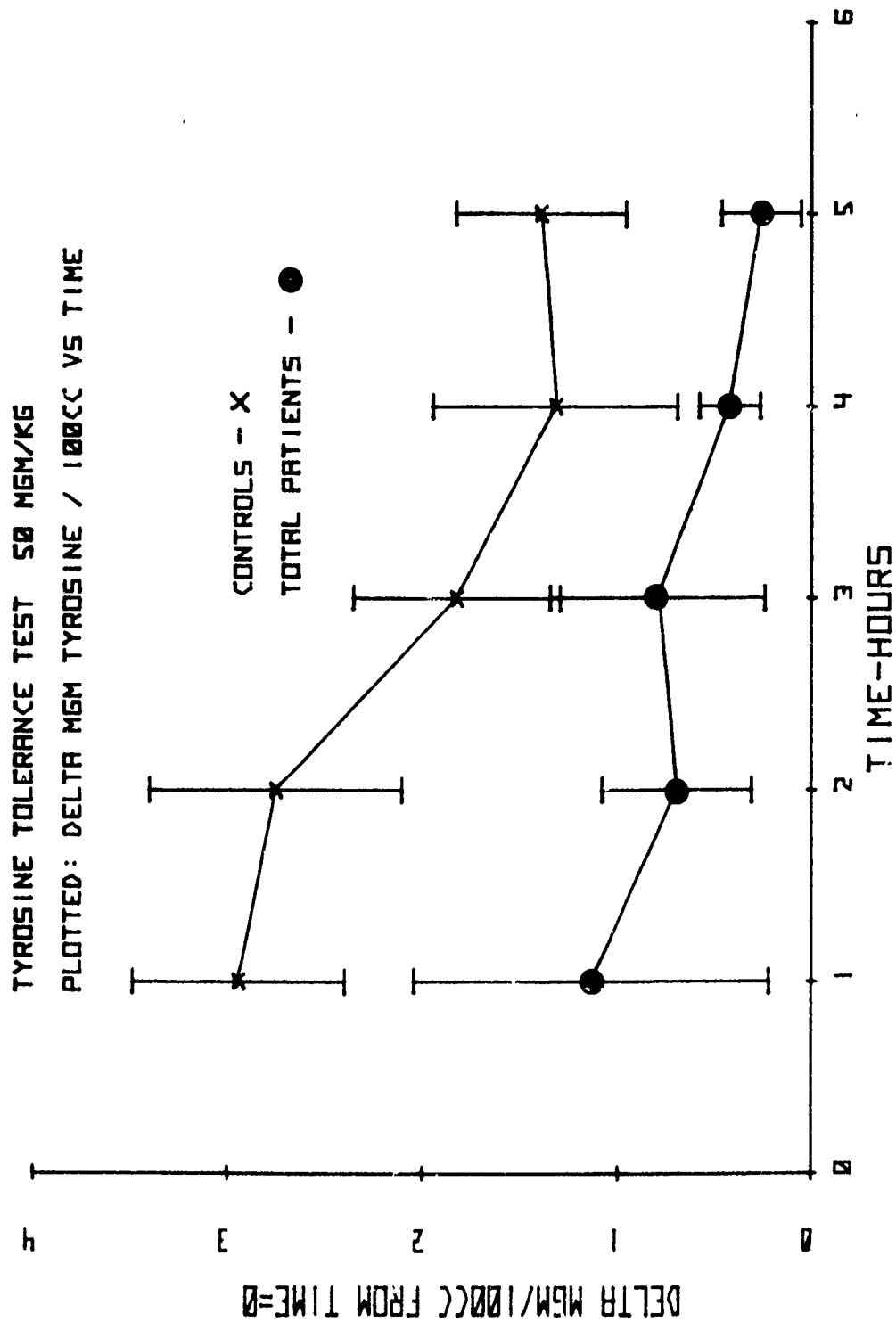


Figure 14

MAJOR PATHWAY FOR CATECHOLAMINE BIOSYNTHESIS:

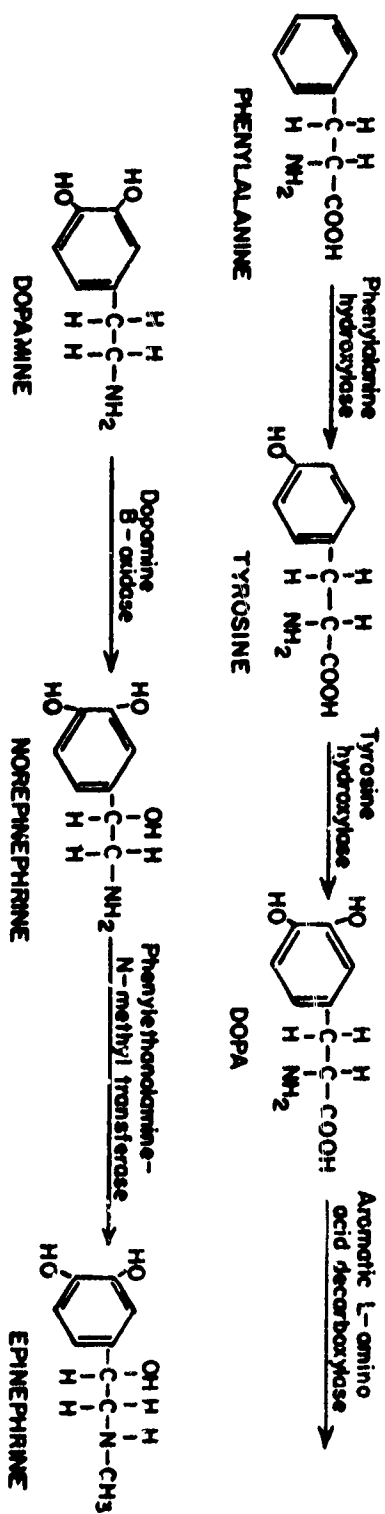


Figure 15

further catabolic marker in the thermally injured.

The tyrosine tolerance curves of the patients showed much smaller increases from fasting than the controls. This indicates either a much greater rate of tyrosine utilization in the burns or, more likely, a failure of absorption of tyrosine. It is interesting that the abnormalities in oral tyrosine tolerance curves observed in burned patients here, closely mimic the previously described abnormalities in tyrosine tolerance curves of myxedematous patients (Fig. 16).²³ The tyrosine appearance-disappearance curves from 100 mg/kg phenylalanine load of uncomplicated burns compared with controls showed a more rapid rate of disappearance of tyrosine in the burns than in the controls. This would indicate that in burns at least this abnormal tyrosine tolerance test is a result of malabsorption.

Decreased absorption of tyrosine and increased flow of phenylalanine in the thermally injured patient would indicate a need for dietary supplementation of these important precursors of catecholamines and thyroid hormone.

PRESENTATIONS AND/OR PUBLICATIONS

None

23. Rivilin RS, Melmon KL, Sjoerdsma A: An oral tyrosine tolerance test in thyrotoxicosis and myxedema. N Engl J Med 272: 1143 (June 3), 1965.

TOLERANCE CURVES AFTER ORAL TYROSINE ADMINISTRATION

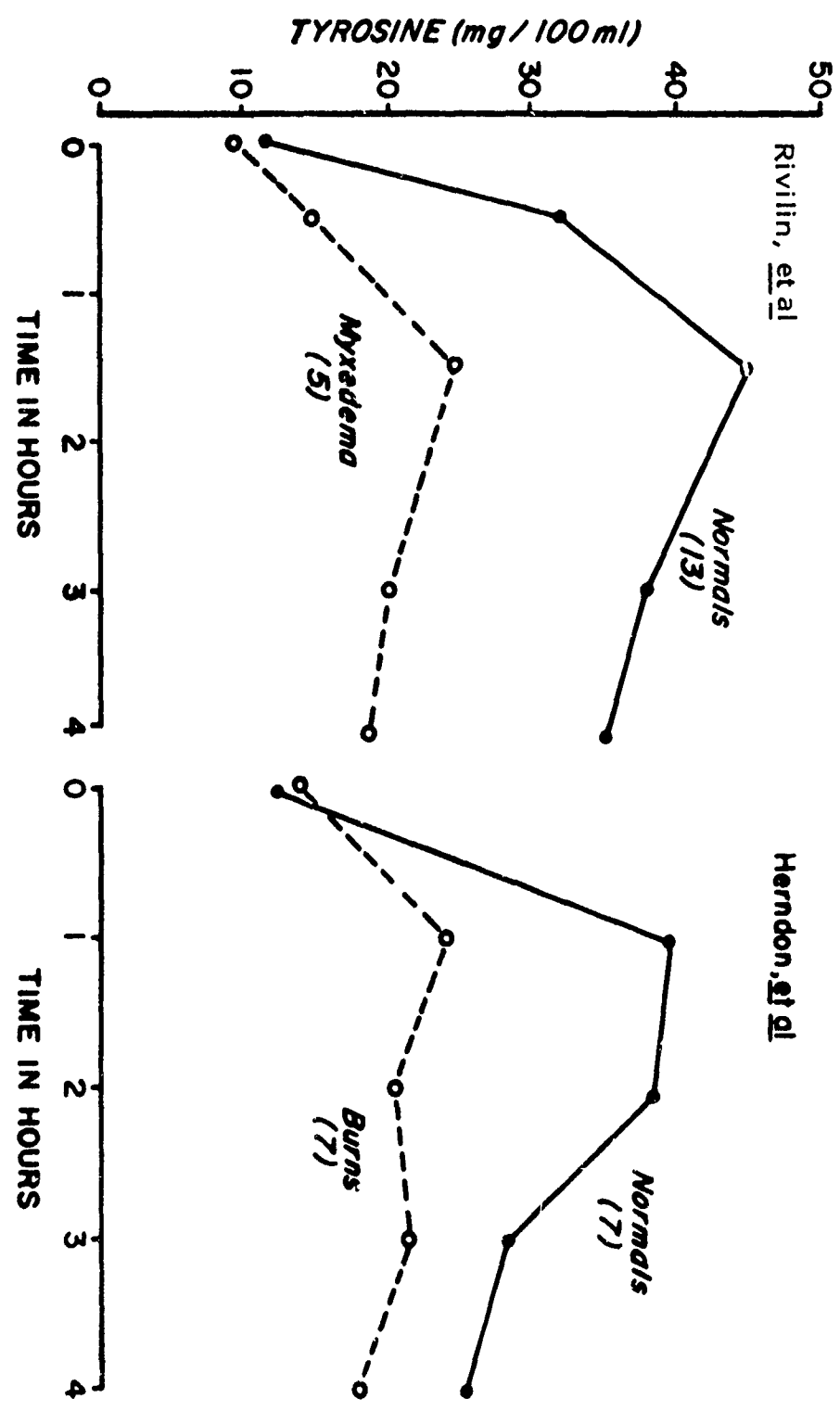


Figure 16

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
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3. DATE PREV. SUMM ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9. SPECIFIC DATA-CONTRACTOR ACCESS ^a	10. LEVEL OF SUM ^a
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10. NO / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
A. PRIMARY	61102A	3A161102B71P		02	069		
B. CONTRIBUTING							
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Evaluation, Donor Site Healing and Mesh Graft Spread With Hyperbaric Oxygen Treatments in Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 01		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
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A. DATES/EFFECTIVE:				FISCAL YEAR		12	
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20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research			
ADDRESS ^a Fort Sam Houston, Texas 78234				ADDRESS ^a Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
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				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Hyperbaric oxygen; (U) Mesh graft; (U) Donor site; (U) Human;							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The objective of this study is to attempt to demonstrate that hyperbaric oxygen shortens the healing time of donor sites and increases the rate of spread of mesh graft applied to suitable recipient areas of burned soldiers.</p> <p>24. (U) The patients were divided into two groups, those receiving hyperbaric oxygen treatments b.i.d. at 2.4 atmospheres of 100% pure oxygen and those not receiving this treatment. In both groups the uniform depth donor site was placed with a Davol dermatome and the time required for healing of this area recorded. A portion of mesh graft was placed on a healthy granulating bed and photografted every two days to observe the filling of the interstices of the mesh.</p> <p>25. (U) 76 01 - 76 09 The patients treated with hyperbaric oxygen healed their documented depth donor sites at a rate equal to or slower than the patients not subjected to hyperbaric treatments. The mesh graft spread in patients not receiving treatments. A separate part of this study showed that documented depth donor sites in volunteers heal at the same rate with hyperbaric oxygen as when untreated. The unescapable conclusion from our work is that hyperbaric oxygen does not accelerate the healing of partial thickness donor sites nor does it speed the healing of mesh graft. Because of the total lack of demonstrable response to hyperbaric oxygen this study was discontinued and the chamber removed from the unit.</p>							

^a Available to contractors upon originator's approval

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

FINAL REPORT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: EVALUATION, DONOR SITE HEALING AND MESH GRAFT SPREAD
WITH HYPERBARIC OXYGEN

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

Hugh D. Peterson, DDS, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY
MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: EVALUATION, DONOR SITE HEALING AND MESH GRAFT SPREAD
WITH HYPERBARIC OXYGEN

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, TX 78234

Period covered in this report: 1 January 1976 - 30 September 1976

Investigators: Hugh D. Peterson, DDS, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Among the many benefits claimed for hyperbaric oxygen in the treatment of burned patients, are decrease in time of donor site healing and increased rapidity of mesh graft spread. These were evaluated on documented depth split thickness skin graft donor sites on convalescent patients requiring grafting and on "mesh" grafts with documented size interstices. The hyperbaric oxygen group were treated with 2.4 atmospheres of 100% oxygen for 60 minutes b.i.d. from the time of graft harvest and placement of graft until healing was complete. No acceleration could be noted when compared with a control group treated in the same fashion but without hyperbaric oxygen treatments. Because of failure to demonstrate any improvement in healing time or mesh graft spread and the difficulty of administering hyperbaric oxygen to patients with large burns, the study was abandoned and the hyperbaric chamber removed from the unit.

Hyperbaric oxygen
Mesh graft
Donor site

EVALUATION, DONOR SITE HEALING AND MESH GRAFT SPREAD WITH HYPERBARIC OXYGEN

There are four purported advantages of administration of hyperbaric oxygen to burn patients; namely, 1) decrease in amount of infection, 2) prevention of conversion of deep second degree burn to third degree burn, i.e., less areas need grafting, 3) decrease in amount of resuscitation fluid needed in the first 48 hours, henceforth less edema and 4) decreased healing time of donor sites and increased rapidity of mesh graft spread. A single person hyperbaric oxygen chamber was obtained and it was elected to first evaluate mesh graft healing and healing of documented depth donor sites. For the initial evaluation small donor sites were placed on either the upper thigh or the forearm of patients requiring grafting procedures. These were done with the Davol dermatome which takes a set thickness graft. The small specimens were pinned on a tongue blade and submitted to pathology for direct measure of the thickness. The donor sites were treated open with fine mesh gauze. The subjects were treated to one hour of 100% oxygen at 2.4 atmospheres twice a day until healing occurred. Healing being defined as separation of the coagulum with total epithelialization. Once the donor site treated with hyperbaric oxygen had healed and separated, a like donor site was placed on the other extremity. Its depth was documented, and it was treated in the same fashion with the exception of the hyperbaric oxygen treatments. Time to healing in the like depth donor sites was 13 to 14 days with and without hyperbaric oxygen. There was no demonstrable decrease in the time of healing.

Donor site healing and mesh graft spread were evaluated in convalescing patients in the 4th to 5th week postburn with wounds of healthy granulation tissue ready for grafting. At the time of grafting a small portion of uniform depth graft was taken with the Davol dermatome and a portion of the skin taken for definitive grafting was also submitted to pathology for measurement of depth. The donor sites were treated in a usual open fashion after placing a single layer of fine mesh upon them. A three inch square area of healthy granulation tissue was picked and a representative portion of the 3:1 meshed graft was placed thereupon with maximum expansion and photographed with a ruler in the field. The meshed graft was then dressed with fine mesh gauze and coarse mesh gauze soaked in 5% Sulf. ylon solution. The patients to be subjected to hyperbaric oxygen were treated in this fashion and then treated with 2.4 atmospheres of 100% oxygen for one hour b.i.d. to include one treatment the day of surgery. Only two patients were in the hyperbaric oxygen group and four in the control group. This is obviously a very small group but since there was absolutely no decrease in healing time of the documented depth donor sites nor any increase in the rapidity of mesh graft spread, the investigators were reluctant to increase the size of the series. If there is significant benefit from treatment with hyperbaric oxygen in increasing healing, it should be demonstrated in a very small series of patients. A difference that would require 50 patients in either group to document statistical significance would be of little clinical value.

Because of our failure to document any improved healing, it was elected to discontinue the hyperbaric oxygen study and return the hyperbaric chamber to the manufacturers.

PUBLICATIONS AND/OR PRESENTATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OF 6393	76 10 01	DD-DR&E(ARM)36	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DIRM INSTR ^a	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
75 07 01	H. TERM	U	U	NA	NL	<input type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO. / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62110A	3A162110A821		00		110	
B. CONTRIBUTING							
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Low Flow Vein Anastomosis in Dogs Anticoagulated With Ancrod: A Model for Blood Vessel Repair in Injured Troops (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 01		75 08		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE				PRECEDING			
B. NUMBER ^a				FISCAL YEAR		18	
C. TYPE:				CURRENT			
D. KIND OF AWARD:				76		.5	
E. AMOUNT:							
F. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				Metabolic Branch			
				ADDRESS: Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: Clement L. Slade, CPT, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-3411			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Willard A. Andes, MD			
				NAME: John W. Sagartz, VD			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Coagulation; (U) Ancrod; (U) Vascular surgery; (U) Dogs							
23. TECHNICAL OBJECTIVE ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Ancrod is a thrombin-like enzyme derived from the venom of the Malayan pit viper. It is a very potent anticoagulant with few undesirable side effects. Vein reconstructions following the penetrating or crush injuries which occur frequently in modern warfare have not been possible with conventional anticoagulants. With Ancrod it may be possible to perform vascular reconstruction in cases which were previously regarded as inoperable. The purpose of this study is to develop a vascular surgical model which is highly predisposed to thrombosis and to utilize this model in the study of Ancrod as a surgical anticoagulant for use in injured soldiers.</p> <p>24. (U) The femoral vein was surgically exposed bilaterally in a group of six adult Mongrel dogs. The vein was then cut transversely and reanastomosed. Venograms were performed at two and four weeks post operatively.</p> <p>25. (U) 75 07 - 75 08 Most of the vein anastomoses remained patent at two weeks postoperatively. Veins which were thrombosed at two weeks had recanalized and were patent by four weeks postoperatively. The dog is thus not an adequate model for these studies because of its active fibrinolytic system and another model will be necessary.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 68
AND 1498 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

TERMINATION

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

**REPORT TITLE: LOW FLOW VEIN ANASTOMOSIS IN DOGS ANTICOAGULATED WITH
ANCROD--A MODEL FOR BLOOD VESSEL REPAIR IN INJURED TROOPS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators

**Clement L. Slade, M.D., Captain, MC
Willard A. Andes, M.D., Major, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: LOW FLOW VEIN ANASTOMOSIS IN DOGS ANTICOAGULATED WITH
ANCROD--A MODEL FOR BLOOD VESSEL REPAIR IN INJURED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Clement L. Slade, M.D., Captain, MC
Willard A. Andes, M.D., Major, MC

Reports Control Symbol MEDDH-288(R1)

Ancrod is a thrombin like enzyme derived from the venom of the Malayan pit viper. This agent is a very potent anticoagulant with few undesirable side effects. It is possible this anticoagulant could be used as an adjunct in vascular surgical procedures which are predisposed to thrombosis. The purpose of this study was to develop a model vascular surgical procedure which was highly predisposed to thrombosis and to use this model in the study of Ancrod as a surgical anticoagulant.

Femoral vein anastomoses were performed bilaterally on six adult mongrel dogs who were not anticoagulated. Follow up venograms at two and four weeks postoperatively, revealed a high rate of patency of the anastomosis. Veins which had clotted at two weeks postoperatively, were found to have recanalized by four weeks postoperatively, therefore the project was terminated. The dog is thus not an adequate model for these studies. It may be necessary to use primates to continue this work.

Coagulation
Ancrod
Vascular surgery
Dogs

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL ^a	
				DA OE 6984	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. ORIGIN INSTR ^a	9. SPECIFIC DATA CONTRACTOR ACCESS ^a	10. LEVEL OF SUMMARY ^a
75 07 01	K. COMP	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
11. NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62110A		3A162110A821		00	
b. CONTRIBUTING						114	
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) A Prospective Comparison Study of Sulfamylon and Silver Sulfadiazine in the Treatment of Burned Troops (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 12		76 09		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				b. NUMBER		c. FUNDS (in thousands)	
EXPIRATION:				7.5		.5	
b. NUMBER:				7T		.2	
c. TYPE:				FISCAL YEAR		4	
d. AMOUNT:				CURRENCY			
e. KIND OF AWARD:				f. CUM. AMT.			
18. RESPONSIBLE DOD ORGANIZATION				19. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Surgical Study Branch Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: Hugh D. Peterson, COL, MC			
TELEPHONE: 512-221-2720				TELEPHONE 512-221-3301			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Arthur D. Mason, Jr, MD			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Topical therapy; (U) Burn injury; (U) Sulfamylon (mafenide); (U) Silvadene (silver sulfadiazine); (U) Humans							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
<p>23. (U) The objective of this study is to compare silver sulfadiazine with Sulfamylon in six groups of burned soldiers, two groups by age 16 to 40, and greater than 40 and three by burn size, 30 to 50 percent, up to 70 percent and greater than 70 percent. The indices to be evaluated are survival, acid base balance, pain on application, bacteriology of the burn wound, pulmonary function, and clinical status of the injured troops.</p> <p>24. (U) Burns greater than 30 percent, seen within the first 72 hours, were randomized by pairs in the aforementioned groups and placed in one of the two agents. The pulmonary functions, acid base balance, wound bacteriology were then followed meticulously until the eschar was entirely separated or until the patient expired.</p> <p>25. (U) 75 07 - 76 09 The study demonstrated that Sulfamylon offered far superior control of the gram-negative population of the burn wound, but had deleterious effects when applied in a regular fashion to burns over 30 percent. The deleterious effects were manifested by respiratory problems, decreased GI function, cerebral changes and earlier death not characterized by sepsis. The study demonstrated that Sulfamylon was most likely not a good agent for initial burn therapy in burns larger than 30 percent but should be reserved for wound problems where it has been very efficacious at this unit. Since the termination of this study silver sulfadiazine has been used as the initial agent we have had increasing problems with the gram-negative flora of the wound and attention is now being turned to an alternating therapy of silver sulfadiazine and Sulfamylon in the early postburn course with careful moderation systemic toxicity to the Sulfamylon.</p>							

FINAL REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

**REPORT TITLE: A PROSPECTIVE COMPARISON STUDY OF SULFAMYLON AND SILVER
SULFADIAZINE IN THE TREATMENT OF BURNED TROOPS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Hugh D. Peterson, DDS, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: A PROSPECTIVE COMPARISON STUDY OF SULFAMYLON AND SILVER SULFADIAZINE IN THE TREATMENT OF BURNED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Hugh D. Peterson, DDS, MD, Colonel, MC
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

During all of the reporting period only silver sulfadiazine was used as the initial topical agent. It was noted to have decreasing efficacy against gram negative organisms mainly Klebsiella. In addition Pseudomonas organisms have been more commonly recovered from the burn wounds, in terminal patients. This decreasing efficacy was highlighted by earlier positive blood cultures and earlier time to death in those patients dying with silver sulfadiazine treatment. Sulfamylon burn cream was again used for wound complications and several patients had wound degeneration which was recouped by subeschar infusion and intermittent application of Sulfamylon burn cream. Sulfamylon burn cream when used early in the burn course was again noted to have a deleterious effect on some patients. Probably the most outstanding thing noted in survivals during this period was that there were only two deaths in the 15 to 40 age group of burns less than 50%. One of these was a patient that entered the hospital with a cerebral death secondary to inhalation injury, the second was a 50% burn that died at 126 days with a nonspecific myocarditis. This low mortality in the 15 to 40 age group in burns of 50% or less contrasts to the 18 deaths in that age group in 1970 and the 14 deaths in that age group in 1973, the last year in which Sulfamylon was used as a primary topical agent. Figures such as this further document the benign quality of silver sulfadiazine in relation to systemic effect on the patient. However this fact is at least in part counter balanced by the poor control of Klebsiella sp.

This study has been terminated because we have continually demonstrated our earlier findings of better bacterial control by Sulfamylon, which must be balanced against its systemic effects in patients with burns of more than 30% of the total body surface. At present we are evaluating a treatment regimen in the early post burn period of 12 hours application of silver sulfadiazine followed by 12 hours application of Sulfamylon alternating in this fashion until the eschar has separated. With this maneuver we have been able to maintain the bacterial density in the eschar at a level of 10^1 to 10^2 less than if only silver sulfadiazine is

used. However an increase in the survival of patients with extensive burns has not been confirmed and we have noted some patients to show the systemic effects of Sulfamylon application. At present we are continuing to pursue this course in patients with burns larger than 50%. The findings of this study suggest that there is probably no ideal topical agent and that emphasis should be placed on surgical removal.

Topical therapy
Sulfamylon (mafenide)
Silvadene (silver sulfadiazine)

Burn injury
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. ORIGIN INSTN ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS ^a	10. LEVEL OF DOW ^a
75 07 01	K.	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO / CODES ^a	PROJECT ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62110A	3A162110A821		00		105	
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Evaluation of the Effect of Fresh-Frozen Plasma on Leukocyte Chemotaxis in Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 09		75 10		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER ^a				FISCAL		4	
c. TYPE.				YEAR		18	
d. AMOUNT:				CURRENCY			
e. YEAR OF AWARD				f. CUM. AMT.			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: James W. Taylor, MAJ, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2943			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: James M. Long, III, MD			
				NAME: Arthur D. Mason, Jr, MD			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Burns; (U) Leukocytes; (U) Chemotaxis; (U) Humans; (U) Plasma							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Evaluation of the in vivo effect of fresh-frozen plasma on leukocyte chemotaxis in thermally injured military personnel.</p> <p>24. (U) Patients with burns over 55% of the total body surface are divided on admission into two groups. One receives the routine resuscitation, the other receives fresh-frozen plasma on colloid during their resuscitation. The functional chemotactic index is determined serially in these patients according to the Warden modification of the Boyden technique namely the ability of leukocytes to migrate through nucleopore filter toward a complement dependent chemotactic, CASEN-SERUM. Other patients who have a low chemotactic index but who had received no fresh-frozen plasma prior to determination, are treated with three units of fresh-frozen plasma per day and the functional chemotactic index determined during the period of treatment with fresh-frozen plasma.</p> <p>25. (U) 75 07 - 75 10 Serial studies of functional chemotactic index (FCI) in 24 burn patients between 3 and 21 days postburn revealed a significantly higher mean FCI (61.4) in those who survived than in those who died (mean FCI 44.7). Twelve patients with low FCI who received fresh-frozen plasma daily showed an increase in mean FCI from 37.7 to 56.0. The changes in patients receiving fresh frozen plasma was not merely an effect of time.</p>							

^a Available to contractors upon originator's approval

FINAL REPORT

PROJECT NO. 3A16211QA821-00, COMBAT SURGERY

**REPORT TITLE: EVALUATION OF THE EFFECT OF FRESH FROZEN PLASMA ON
LEUKOCYTE CHEMOTAXIS IN BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**James W. Taylor, M.D., Major, MC
James M. Long, III, M.D., Lieutenant Colonel, MC
Arthur D. Mason, Jr. M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF THE EFFECT OF FRESH-FROZEN PLASMA ON
LEUKOCYTE CHEMOTAXIS IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: James W. Taylor, M.D., Major, MC
James M. Long II, M.D., LTC, MC
Arthur D. Mascn, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288 (R1)

Warden and his associates reported that leukocytes from burned patients showed decreased chemotaxis and that after 72 hours post burn the impairment of leukocyte chemotaxis is directly correlated with the clinical status of the patient and is highly predictive for ultimate mortality, and that in vitro normal serum can restore chemotactic function of the granulocytes from burned patients to normal.

In the present study, chemotaxis of leukocytes from burned patients was studied using a modified Boyden chamber following the methods described by Warden. All studies were performed in triplicate with the person counting the cells blinded as to the source of the specimen. Eighty-four determinations of the Functional Chemotactic Index (FCI) were made on 24 burned patients between the third and twenty-first post burn day. Those burned patients who ultimately survived showed a mean FCI of 61.4 (range 19.7 to 118) compared to 44.7 (range 11 to 100.7) for the nonsurvivors ($p < 0.01$).

Subsequently, 12 burned patients who were first demonstrated to have a low FCI were treated with three units of fresh frozen plasma per day, and the FCI was determined serially. The mean FCI following treatment was 56.0 (range 36.2 to 93.9) compared to 37.7 (range 15.4 to 68.4) before treatment ($p < 0.01$). Comparison of this group with similar patients from our earlier series who received no fresh frozen plasma showed that this was not merely an effect of time.

This study reconfirmed the findings that ultimate mortality in burned patients is associated with significant depression of the FCI and demonstrated that leukocyte chemotaxis in burned patients can be enhanced in vivo by some constituent of fresh frozen plasma.

Burns
Leukocytes

Chemotaxis
Humans

Plasma

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY					1. AGENCY ACCESSION ^a		2. DATE OF SUMMARY ^a		REPORT CONTROL SYMBOL DD-DR&E(AR)434	
3. DATE PREV SUMMARY		4. KIND OF SUMMARY		5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. RESEARCH ^a	8. SUBJECT ^a	9. SPECIFIC DATA- CONTRACTOR ACCESS		10. LEVEL OF SUMMARY
75 07 01		H. TERM		U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		A. WORK UNIT
11. NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER		
A. PRIMARY		62110A		3A162110A821		00		107		
B. CONTRIBUTING										
C. CONTRIBUTING										
11. TITLE (Precede with Security Classification Code) (U) Evaluation of Gastric Physiologic Disturbances Associated With Thermal Injury in a Military Population (44)										
12. SCIENTIFIC AND TECHNOLOGICAL AREA ^a 003500 Clinical Medicine										
13. START DATE			14. ESTIMATED COMPLETION DATE			15. FUNDING AGENCY			16. PERFORMANCE METHOD	
72 01			76 09			DA			C. In-House	
17. CONTRACT GRANT Not Applicable						18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS		20. FUNDS (in thousands)
A. DATE/EFFECTIVE						PRECEDENCE		.5		16
B. NUMBER ^a						FISCAL YEAR		.2		6
C. TYPE:						CURRENCY				
D. KIND OF AWARD:						E. AMOUNT:				
21. RESPONSIBLE DOD ORGANIZATION						22. PERFORMING ORGANIZATION				
NAME ^a US Army Institute of Surgical Research						NAME ^a US Army Institute of Surgical Research				
ADDRESS ^a Fort Sam Houston, Texas 78234						ADDRESS ^a Fort Sam Houston, Texas 78234				
RESPONSIBLE INDIVIDUAL						PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)				
NAME Basil A. Pruitt, Jr, COL, MC						NAME ^a David K. Teegarden, MAJ, MC				
TELEPHONE: 512-221-2720						TELEPHONE: 512-221-6532				
23. GENERAL USE						SOCIAL SECURITY ACCOUNT NUMBER:				
FOREIGN INTELLIGENCE NOT CONSIDERED						ASSOCIATE INVESTIGATORS				
						NAME: Basil A. Pruitt, Jr, COL, MC				
						NAME: Albert J. Czaja, M.D. DA				
24. KEYWORDS (Precede EACH with Security Classification Code) (U) Thermal injury; (U) Burn patients; (U) Evaluation; (U) Gastric physiologic Disturbances										
25. TECHNICAL OBJECTIVE ^a 26. APPROACH. 27. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)										
23. (U) To study the gastric pathophysiology of the thermally injured soldier, so as to better define etiologic factors responsible for Curling's ulcer.										
24. (U) Evaluation to be carried out on thermally injured soldiers with greater than 30% TBS area injury admitted to the USAISR. Study will be stratified so that a group of patients in the 30 to 50% TBS area injury and the second group in the 50 to 100% TBS area injury will be included. Investigative procedures will be performed within 24 hours if possible and at 72 hours postburn. Burns of greater than 50% body surface area will also be studied at 5 to 7 days post injury and all patients will then be studied between the 9th and 12th day post injury and at 30 days or discharge. Studies will encompass: (1) gastric endoscopy with photography and biopsy for semiquantitative mucous determination, (2) ion flux across the gastric mucosa, (3) coagulation studies, (4) measurements of gastric clearance of radioactive isotopes, and (5) evaluation of the role of bacteremia.										
25. (U) 75 07 - 76 09 Early gastritic and duodenitic changes are present in 7-80% of patients with burns of 40% or more of the body surface. Gastric acid production is increased markedly in those patients who develop clinically significant complications of Curling's ulcer. Gastrin level assay suggests an intact gastrin control mechanism. Prophylactic antacid therapy reduces bleeding from this variant of stress ulcer disease.										

^aAvailable to contractors upon originator's approval.

TERMINATION REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

**REPORT TITLE: EVALUATION OF GASTRIC PHYSIOLOGIC DISTURBANCES ASSOCIATED
WITH THERMAL INJURY IN A MILITARY POPULATION**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**David K. Teegarden, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF GASTRIC PHYSIOLOGIC DISTURBANCES ASSOCIATED
WITH THERMAL INJURY IN A MILITARY POPULATION

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: David K. Teegarden, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Endoscopic abnormalities of the gastroduodenal mucosa develop in 78% of thermally injured patients with burns involving 25% or greater of the total body surface.

Physiologic studies revealed that production of gastric mucosubstances was normal and not etiologically significant. Increased permeability of the gastric mucosa to hydrogen ions was not consistently noted in those with endogenous abnormalities but correlated with clinical progression of disease.

Current studies include evaluation of serum pepsinogen as a predictor of gastroduodenal mucosal disease, duodenopyloric reflux of bile salts as an etiologic factor in stress ulcers, and evaluation of cimetidine as a therapeutic agent in prevention of stress ulcers following thermal injury.

Evaluation
Gastric physiologic disturbances
Thermal injury
Burn patients

EVALUATION OF GASTRIC PHYSIOLOGIC DISTURBANCES ASSOCIATED WITH THERMAL INJURY IN A MILITARY POPULATION

Over the past several years, gastrophysiologic studies have been performed in the thermally injured soldier to determine those factors that may be important in the etiology of erosive and ulcerative disease of the stomach and duodenum. Patients with a greater than 25% total body surface burn were at most risk to develop gastroduodenal complications and were included in the physiologic studies.

Gastroduodenal Endoscopy.

Endoscopy of the esophagus, stomach, and duodenum was performed in a serial fashion. Seventy seven thermally injured patients were included in the study and ranged in ages from 16 to 74. Superficial gastric mucosal injury was noted in approximately 87% of the patients. The gastric lesions were most often noted in the fundus and body of the stomach, and the antrum was less often involved but was abnormal in one third of the patients. Discrete ulcerative disease was found in 26.5% of the patients, and, of these, six had concomitant duodenal ulcers. Duodenal inflammation was found in 81% of those patients, and was noted to be present only in those patients with a greater than 35% total body surface burn. Duodenitis was the most frequently encountered diagnosis, and was defined by the appearance of erythema, edema, and increased mucosal friability. In two patients, ulcerative disease was seen to evolve from superficial erosive disease. Discrete duodenal ulceration was encountered in 26% of the patients, and all were noted to have accompanying duodenitis.

It can be concluded from this endoscopic evaluation that superficial gastroduodenal mucosal injury is a frequent accompaniment of severe thermal injury. This study emphasizes that this disease is a diffuse process and involves many portions of the gastroduodenal mucosa. Discrete ulceration in either the stomach or duodenum was not present unless accompanying superficial inflammatory changes were noted around the ulceration.

Physiologic Studies

Histochemical techniques were employed to study the mucous protective barrier of the stomach. It has been postulated in previous literature that a decrease in mucus production, or a change in its characteristics, might render the gastric mucosal membrane more susceptible to damage. Of the nine patients studied, all were noted to have a normal production of gastric mucus. It was therefore concluded that mucus substances produced in the stomach are not an important etiologic factor in the development of acute gastroduodenal stress ulceration following thermal injury.

A second study utilized the lithium flux technique to evaluate back-diffusion of the hydrogen ion in the gastric mucosa and the integrity of the gastric mucosal barrier. Our data documented that increased back diffusion of hydrogen ions was not an etiologic factor in the early development of gastric lesions following thermal injury. However, disruption of the gastric mucosal barrier seemed to correlate with endoscopic and clinical progression of mucosal disease, suggesting that hydrogen ion is an important contributing factor in the progression of disease and perhaps is useful as a prognostic index.

Current Physiologic Studies in Progress

Little attention has previously been given to the role of pepsin digestion of the gastric mucosa following thermal injury. Currently, we are utilizing a radioimmunoassay of serum pepsinogen as a reflector of gastroduodenal morphology. A pilot study has been performed, and indicates that serum pepsinogen levels rise markedly prior to gastroduodenal bleeding in two of 11 patients studied. Although the numbers are small, we are currently evaluating the endoscopic appearance of the gastroduodenal mucosa and determining if correlations exist between the serum pepsinogen and the endoscopic appearance of the stomach.

In the experimental animal, disruption of the gastric mucosal membrane can be accomplished easily by topical application to the gastric mucosa of substances that have detergent properties, such as bile, aspirin, urea, etc. Reflux of bile through the pylorus into the stomach has been postulated as an etiologic factor in the development of erosive and ulcerative disease of the stomach. We currently are using a steroid dehydrogenase enzymatic assay to quantitate bile reflux early in the postburn period. Bile reflux will be compared between thermally injured and normal controls and will be correlated with endoscopic findings.

Since the presence of hydrochloric acid is essential in the experimental production of stress ulceration, control of hydrogen ion secretion by the gastric mucosa would seem a logical method of treatment. Histamine is felt to be the final common pathway in gastric acid secretion regardless of the stimulus employed. A new group of drugs has been developed which antagonizes the action of histamine on the parietal cells of the gastric mucosa. Cimetidine is a third generation histamine antagonist, and is being given in a double-blind fashion to determine its effectiveness in prevention of gastroduodenal disease. This study is currently in progress.

Summary

Previous gastrophysiologic studies have been extensive in this unit, yet fail to define the etiologic factors solely responsible for Curling's ulcer. Future studies, as outlined above, are underway to

further define the pathophysiologic events important in this disease process. In addition, various therapeutic modalities are being studied to determine the most effective in the treatment of this disease process.

PRESENTATIONS

None

PUBLICATIONS

1. Czaja AJ, McAlhany JC, Jr., Pruitt BA Jr: Acute gastroduodenal disease following thermal injury: an endoscopic evaluation of incidence and natural history. *New Eng J Med* 291:925-929, 1974.
2. McAlhany JC Jr, Czaja AJ, Cathcart RS III, Spicer SS, Foley FD, Pruitt BA Jr: Histochemical study of gastric mucosubstances after thermal injury: correlation with endoscopic evidence of acute gastroduodenal disease. *J Trauma* 15:609-612, 1976.
3. McAlhany JC Jr, Czaja AJ, Villareal Y, Mason AD Jr, Pruitt BA Jr: The gastric mucosal barrier in thermally injured patients: correlation with gastroduodenal endoscopy. *Surg Forum* 25:414-416, 1974.
4. Czaja AJ, McAlhany JC Jr, Andes WA, Pruitt BA Jr: Pathogenesis of acute duodenal disease after burns: role of acid secretion. *Gastroenterology* 68:1023, 1975.
5. Czaja AJ, McAlhany JC, Pruitt BA Jr: Acute duodenitis and duodenal ulceration after burns. Clinical and Pathological characteristics. *JAMA* 232:621-624, 1975.
6. Czaja AJ, McAlhany JC, Pruitt BA Jr: Gastric acid secretion and acute gastroduodenal disease after burns. *Arch Surg* 111:243-245, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION ^a	2 DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3 DATE PREV SUM ^a	4 KIND OF SUMMARY	5 SUMMARY SCTY ^a	6 WORK SECURITY ^a	7 REGRADING ^a	8a DISEN INSTR ^a	8b SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
75 07 01	K. COMP	U	U	NA	NL	<input type="checkbox"/> YES <input type="checkbox"/> NO	
10 NO / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62110A	3A162110A821	00	109			
b. CONTRIBUTING							
c. CONTRIBUTING							
11 TITLE (Precede with Security Classification Code) ^a (U) The Zinc Requirements of the Burned Rat and the Influence of Zinc on LDH Activity, Growth Rate and Wound Healing: A Model of Burned Soldiers (44)							
12 SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13 START DATE		14 ESTIMATED COMPLETION DATE		15 FUNDING AGENCY		16 PERFORMANCE METHOD	
73 11		76 09		DA		C. In-House	
17 CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19 PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER:				FISCAL YEAR		8	
c. TYPE:				CURRENT			
d. KIND OF AWARD:				e. CUM. AMT.			
19 RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Laboratory Animal Branch Fort Sam Houston, Texas 78234			
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TELEPHONE: 512-221-2720				TELEPHONE 512-221-4951			
21. SPECIAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Ysidro Villarreal, BS			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Zinc requirements; (U) Burns; (U) Wound healing; (U) Lactate dehydrogenase; (U) Rats							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number Precede text of each with Security Classification Code.)							
23. (U) To determine the effect of zinc dosage on plasma zinc levels and the growth rate of burned rats as an animal model for the zinc requirements of burned soldiers.							
24. (U) Groups of scald burned rats (60% total body surface) will be fed a zinc free diet and administered daily doses of zinc acetate intraperitoneally. The animals will be weighed and observed for signs of zinc deficiency. Plasma zinc will be determined at 10 days postburn and the animals euthanized.							
25. (U) 75 07 - 76 09 No signs of zinc deficiency were seen in the burned animals. No significant differences were found in the plasma zinc or weight gain of the animals receiving zinc over those receiving no zinc. Weight gain was not improved with zinc administration.							

^a Available to contractors upon originator's approval

DD FORM 1498
1 MAR 65

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1488A 1 NOV 65 AND 1498 1 MAR 65 (FOR ARMY USE) ARE OBSOLETE

FINAL REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

**REPORT TITLE: THE ZINC REQUIREMENTS OF THE BURNED RAT AND THE
INFLUENCE OF ZINC ON LDH ACTIVITY, GROWTH RATE
AND WOUND HEALING: A MODEL OF BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Donald J. Johnson, DVM, Major, VC
Ysidro Villarreal, BS
Harrel L. Walker, MS
Arthur D. Mason, Jr., MD**

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A152110A821-00, COMBAT SURGERY

REPORT TITLE: THE ZINC REQUIREMENTS OF THE BURNED RAT AND THE
INFLUENCE OF ZINC ON LDH ACTIVITY, GROWTH RATE
AND WOUND HEALING: A MODEL OF BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Donald J. Johrison, DVM, Major, MC
Ysidro Villarreal, BS
Harrel L. Walker, MS
Arthur D. Mason, Jr, MD

Report Control Symbol MEDDH-288 (R1)

The objective of this study is to determine if rats with extensive scald burns have an increased requirement for the trace metal zinc. Rats were scald burned over 60% of their total body surface and administered parenteral zinc at 0, 50, and 100 micrograms per day. The animals were fed a zinc-free diet to minimize the intake of exogenous zinc. The animals were weighed preburn and at 7 and 10 days postburn. Plasma zinc levels were determined at 10 days postburn.

No significant differences were found in the postburn weights or the plasma zinc levels of animals treated with zinc and those with no zinc source. No lesions of zinc deficiency were seen.

Zinc requirements
Burns
Wound healing
Lactate dehydrogenase
Rats

THE ZINC REQUIREMENTS OF THE BURNED RAT AND THE INFLUENCE OF ZINC LDH ACTIVITY, GROWTH RATE AND WOUND HEALING: A MODEL OF BURNED SOLDIERS

The zinc requirement of man is not fully known and the reported cases of zinc deficiency are rare. A zinc deficiency syndrome in man was reported in 1963 by Prasad in Egyptian males with a dwarfing and hypogonadal syndrome (1). Zinc has recently been reported to be essential in vitamin A metabolism. Depressed vitamin A levels in plasma were found in zinc deficient rats and in children with low plasma zinc (2). The low vitamin A levels may account for the epithelial lesions seen in zinc deficient rats and other animals.

Altered zinc levels have been reported in burn patients (3). Previously we have found low zinc levels in the plasma of burned rats with 60% total body surface burns (TBS). The tissue levels of zinc did not correlate with zinc dosage or time post burn. Lactic dehydrogenase activity was only altered during the first 48 hours post burn.

METHODS

Male, Sprague-Dawley rats weighing between 172-200 grams were used in this study. All animals were anesthetized with pentobarbital sodium intraperitoneally (0.6 mg/100 gm body weight) and the hair clipped on the dorsum of the back and the abdomen. The animals in all groups were 3⁰ scald burned over 60% TBS by the method of Walker and Mason (4). All animals were fed a zinc-free diet (Nutritional Biochemical Co.) *ad libitum*, housed in stainless steel wire bottomed cages, and given water *ad libitum*. Fifty or 100 ug of zinc, as zinc acetate in saline, was administered daily intraperitoneally. Normal saline was administered to the zinc deficient animal group intraperitoneally once daily. The animals were weighed on the day of burning and at 7 and 10 days post burn. The animals were then anesthetized with chloroform to obtain a blood sample by intracardiac puncture. The blood was heparinized and plastic tubes used to store and centrifuge the samples. Plasma zinc was determined by atomic absorption spectrophotometry using a model AA-5 Varian Techtron.

1. Prasad AS, Miale A, Jr, et al: Biochemical studies in dwarfism, hypogonadism and anemia. Arch Int Med 111: 407, 1963.

2. Smith JC, et al: Zinc: A trace element essential in Vitamin A metabolism. Science 181: 954-955, 1973.

3. Larson DL, Maxwekk R, Abston S, Dobrkovsky M: Zinc deficiency in burned children. Plas Reconstr Surg 46: 13-21, 1970.

4. Walker HL, Mason AD, Jr: A standard animal burn. J Trauma 8: 1049-1051, 1968.

RESULTS

The plasma zinc levels are shown in Table 1. Blood samples from 8 animals are in each group. The mean plasma zinc levels did not differ significantly between groups using analysis of variance (ANOVA). Four of 8 samples in the zinc-free group and 3 of 8 samples in the 50 ug/day zinc had plasma zinc levels below 100 ug/100 ml plasma. All animals receiving 100 ug zinc daily had plasma zinc levels in excess of 100 ug/100 ml plasma. 100 ug/100 ml is considered the low normal value for the rat and man.

Table 1. Mean Plasma Zinc Levels at 10 Days Postburn

Group	Treatment ug Zinc/Day	Mean ± S.E	Range
I	0	109.9 ± 12*	60 - 165
II	50	111.3 ± 11	70 - 160
III	100	142.5 ± 28	110 - 185

* All values are ug/100 ml plasma

The body weights are shown in Table 2. The animals lost weight between burning and the 7th postburn day. The administration of zinc did not result in any diminution of the weight loss which occurs early in the post burn period. The loss of weight did not differ significantly between groups. Between the 7th and 10th post burn day, the animals began to gain weight at a rate normal for unburned animals of from 3-6 gm daily. At 10 days post burn, the weights between groups did not differ significantly.

Table 2. Mean Weight (grams ± S.E.)

Group	Treatment ug/Zinc/Day	0	Days Postburn 7	10
I	0	183.6 ± 1.04	167.3 ± 2.4	187.0 ± 2.4
II	50	184.3 ± 1.94	171.6 ± 2.4	186.5 ± 3.8
III	100	182.6 ± 1.44	164.6 ± 2.2	181.8 ± 2.6

DISCUSSION AND SUMMARY

The absence of lesions of zinc deficiency in this study and earlier studies in which animals were observed for 30 days postburn on zinc-free diets and without exogenous zinc indicates that zinc is conserved by the animals and that large zinc losses are not occurring through the burn wound and urine. The rats eat their eschar as it separates which serves as a zinc source for the animal which the burn patient would not have.

The mean plasma zinc levels in 47 unburned animals from our rat colony is 150.6 ± 12.8 ug/100 ml. Individual values range from 100 - 231 ug/100 ml plasma. In a test of the diet, a group of 5 unburned animals fed the zinc-free diet for 4 months had plasma zinc levels of 37.5 - 87.5 ug/100 ml. Three of the 5 animals had lesions of zinc deficiency and in the one animal which had no lesions, the plasma zinc level was 87.5 ug/100 ml. In plasma, one-third of the zinc is firmly bound to globulin and the remaining two-thirds is loosely bound to albumen (5). The unburned animals fed the test diet for 4 months and with lesions of zinc deficiency had plasma zinc levels compatible with a complete depletion of the loosely bound zinc. In the present study, the plasma zinc determinations were all above values which would indicate a complete depletion of the loosely bound zinc.

The age and weight of the rat when deprived of zinc may be important to produce lesions of zinc deficiency. We have not seen an immediate rapid decline in weight gain or plasma zinc in unburned animals weighing 180-200 gm. Most investigators using rats for nutritional studies use weanling rats which weigh approximately 45 gm. Animals of this age and weight do not have a large zinc pool to maintain plasma and tissue zinc levels above the minimum required for normal growth and the enzymatic processes for which zinc is essential.

The negative results in producing a zinc deficiency syndrome in burned rats indicates that 180-200 gm rats have sufficient reserves to prevent complete depletion of zinc in the critical early post burn period. The administration of exogenous zinc in excess of daily requirements did not significantly alter changes in the plasma zinc levels or the weight loss and gain in the early post burn period.

PUBLICATIONS AND/OR PRESENTATIONS

None

5. Prasad AS (ed): Zinc Metabolism. Charles C. Thomas, Springfield; Ill., 1966.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OG 6967	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DRG'S INSTR ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS ^a	10. LEVEL OF SUM ^a
76 10 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
11. NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62110A		3A162110A821		00	
b. CONTRIBUTING						105	
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) The Relationship Between Limb Blood Flow and Metabolism Following Thermal Injury in Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 02		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE ^a		19. PROFESSIONAL MAN YRS	
Not Applicable				76		1.0	
a. DATES/EFFECTIVE:				7T		.3	
b. NUMBER:				FISCAL YEAR		CURRENT	
c. TYPE:				4. AMOUNT:		35	
d. KIND OF AWARD:				f. CUM. AMT.		11	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
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22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
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				NAME: Douglas W. Wilmore, MD			
				NAME: Arthur D. Mason, Jr, MD DA			
23. REVENUES (Precede Each with Security Classification Code) (U) Cardiac output; (U) Glucose; (U) Pyruvate; (U) Humans (U) Plethysmography; (U) Limb blood flow; (U) Mean skin and body temperatures							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
23. (U) To determine in rates of blood flow and metabolism and relate them to (a) extent of thermal injury, (b) cardiac output; (c) thermal equilibrium, and (d) general level of total body metabolism of burned soldiers (8-10) and normal controls.							
24. (U) Limb Blood Flow via venous occlusion plethysmography usually of total leg but arms may also be used with selected patients.							
Cardiac Output - Indicator (cardiogreen) dilution							
Total Body Metabolism - Indirect calorimetry via collection of expired air in Douglas bags and analysis on mass spectrophotometer.							
Arteriovenous sampling across limb - Analysis for oxygen consumption and carbon dioxide production as well as uptake of glucose and FFA and release of pyruvate and lactate.							
Body Temperatures - Rectal and several skin temperatures will be monitored by copper-constantan thermocouples.							
General Protocol - After post-absorptive subject at thermal equilibrium in quiet, warm (29°C) room, his resting metabolic rate and body temperatures will be determined. The limb to be studied will then be placed into a plethysmograph and 8-10 limb blood flow measurements taken. This will be followed by cardiac output determinations (3) and arteriovenous blood sampling whenever feasible, limb blood flow measurements will be repeated. Total experimental time estimated at two to three hours.							
25. (U) 76 02 - 76 09 Blood flow is increased only to the burned legs and the close relationship between leg blood flow and the size of leg burn suggest that most of the increased peripheral blood flow is directed to the wound while the extent of local injury is the major determinant of limb flow. Temperature changes of the wound will modify leg blood flow in a predictable manner - increasing temperature, cause high flows and vice versa. Leg oxygen consumption and substrate turnover, total body blood flow and energy metabolism are just underway and not enough data is available for comment.							

TERMINATION REPORT

PROJECT NO, 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE RELATIONSHIP BETWEEN LIMB BLOOD FLOW AND METABOLISM
FOLLOWING THERMAL INJURY IN SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 July 1975 - 30 September 1976

Investigators:

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Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE RELATIONSHIP BETWEEN LIMB BLOOD FLOW AND METABOLISM
FOLLOWING THERMAL INJURY IN SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: Louis H. Aulick, Ph.D., Major, MSC
Douglas W. Wilmore, M.D.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Simultaneous measurements of resting leg blood flow and surface and rectal temperatures were performed in 45 studies of nine normals and 28 burn patients. The patients had burns from 3-86% of the total body surface with leg injury ranging from 0-87.5% of the leg surface.

In the patient group, blood flow was essentially normal in the uninjured legs, increased in a curvilinear manner with the size of the leg burn, and approached a plateau of 8.0 ml/100 ml·min as the per cent of leg burn exceeded 60%. Increasing leg surface temperature by 5°C increased blood flow to the same extent in patients with burned and unburned extremities as it did in normals. Peripheral blood flow is increased following thermal injury.

Peripheral circulation
wound blood flow
Thermal injury

THE RELATIONSHIP BETWEEN LIMB BLOOD FLOW AND METABOLISM FOLLOWING THERMAL INJURY

Following successful resuscitation of burn shock, cardiac output rises above normal resting levels. The increase in total circulation is closely related to the per cent of the total body surface burned, accompanies an increase in resting metabolic rate, and may reach levels 2-3 times normal (1,2).

Indirect evidence suggests that much of the extra blood flow in the burned patient is directed to peripheral tissues. Gump, et al (3), found that splanchnic blood flow represented a smaller portion of the cardiac output in three severely burned patients than it did in normal individuals or patients with postoperative infection. Wilmore and collaborators (4) suggested that much of the elevated peripheral blood flow is directed to the skin. They found that burned patients maintained above normal surface temperatures despite increased evaporative cooling of the wound, and concluded that this elevated skin temperature could be the result of increased superficial blood flow. In addition, the coefficient of core-to-skin heat conductance, an index of skin blood flow, was twice normal in burn patients in a variety of thermal environments.

While these heat balance studies tend to support the concept of increased peripheral (surface) blood flow in the burned patient, the influence of the burn wound on the distribution of this elevated peripheral flow is unknown. Through the use of venous occlusion plethysmography, this study provides the first measurements of peripheral blood flow in thermally injured patients.

1. Baxter CR: Fluid volume and electrolyte changes of the early postburn period. In: Clinics in Plastic Surgery (Vol. 1, No. 4), edited by J. A. Moncrief, Philadelphia, Pa.: W. B. Saunders, 1974, p. 693-709.

2. Unger A, Haynes BW Jr: Hemodynamic studies in severely burned patients. Surg Forum 10:356-361, 1959.

3. Gump FE, Price JB Jr, Kinney JM: Blood flow and oxygen consumption in patients with severe burns. Surg Gynec Obstet 120: 23-28, 1970.

4. Wilmore DW, Mason AD Jr, Johnson DW, Pruitt BA Jr: Effect of ambient temperature on heat production and heat loss in burn patients. J Appl Physiol 38:593-597, 1975.

MATERIALS AND METHODS

Subjects

Twenty eight thermally injured patients, with a mean burn size of 39.5% of total body surface, range 3-86%, and nine normal individuals were studied. The subjects' ages ranged from 15-55 years. Patients selected for this study were 1) free of any pre-existing disease prior to injury; 2) hemodynamically stable after an uneventful resuscitation; 3) in a normal state of hydration with a hematocrit greater than 33 and without abnormalities in serum electrolyte concentrations, osmolality, or pH; 4) free of systemic infection as determined by clinical symptoms and signs and daily blood cultures; 5) 6-21 days postinjury, before significant healing had occurred; and 6) able to participate in the study. Patients represented a wide range of thermal injury, in relation to both the size of the total body surface burn (%TBS) and the extent of leg injury (%LB). The wounds were treated by a variety of techniques. The majority of patients were treated by the exposure method and received topical applications of a silver sulfadiazine cream (Silvadene^R cream) to the injured surface, but some were treated with 11% mafenide acetate topical burn cream (Sulfamylon^R cream), saline, or 5% mafenide-saturated dressings, 0.5% silver nitrate soaks, or cutaneous allograft biological dressings. The last treatment of the wound was at least 8 hours prior to study. In the nine patients treated by closed techniques, the dressings were removed only from the left leg.

Study Design

All experiments took place in the environmental chamber described previously (4). Room temperature was maintained at 30°C, and relative humidity ranged between 40-50%. Control subjects were studied in shorts or shorts and halter, and patients were similarly draped with light cotton towels. The postabsorptive subject was moved to the study room in the early morning and placed in bed. Water was given on request and, in some patients, intravenous infusion of 0.04 molar sodium chloride solution was maintained to insure normal hydration. Nine copper-constantan thermocouples were attached to the skin at the same sites for all

4. Wilmore DW, Mason AD Jr, Johnson DW, Pruitt BA Jr: Effect of ambient temperature on heat production and heat loss in burn patients. J Appl Physiol 33:593-597, 1975.

subjects (dorsum of foot, lateral and posterior calf, posterior and anterior thigh, dorsum of hand, forearm, abdomen and low back). Leg skin temperatures were monitored from both legs, using five additional thermocouples, in patients with asymmetrical leg burns. In those patients treated in dressings, the thermocouples were placed on the wound, under the dressing. A rectal probe was inserted to a distance of 10 cm from the external anal sphincter. All temperatures were recorded at 5-minute intervals throughout the study. After the subject had rested quietly for at least one hour, the left leg was inserted into a soft, pliable, water-impermeable boot, and then placed in a full-length plethysmograph. Water was added to the plethysmograph at a temperature equal to the mean leg skin temperature, and maintained at this temperature through a 30-minute equilibration period and the following 8-10 blood flow measurements. A 2-3 minute interval separated the blood flow determinations.

In selected studies, water temperature was then raised 5°C above neutral bath temperature and, following 30 minutes of equilibration, a second set of leg blood flow measurements was performed. At the end of each study, the plethysmograph was drained and the water volume determined. Total time for each study was less than three hours, and the subjects usually slept throughout the study.

Leg Blood Flow Measurements

The validity (5,6,7), simplicity, and noninvasive character of venous occlusion plethysmography make this the optimal approach to the study of peripheral blood flow. The plethysmograph used in this study is basically a rectangular box of 6.4 mm plexiglass, slightly tapered toward the foot, with an overall length of 86 cm and a volume of 51.9 liters (Fig. 1). It consists of three sections: the thigh plate assembly with supporting rings, a full length top, and a "trough" section made up of the floor, two sides, and distal wall. A rubber gasket covered with high vacuum stopcock grease is used to obtain a watertight seal when these sections are bolted together. The top is raised at the distal end to accommodate the foot. On this elevated portion of the top section is

5. Formel PF, Doyle JT: Rationale of venous occlusion plethysmography. *Circ Research* 5:354-357, 1957.

6. Greenfield ADM, Whitney RJ, Mowbray JF: Methods for the investigation of peripheral blood flow. *Brit Med Bull* 19:101-109, 1963.

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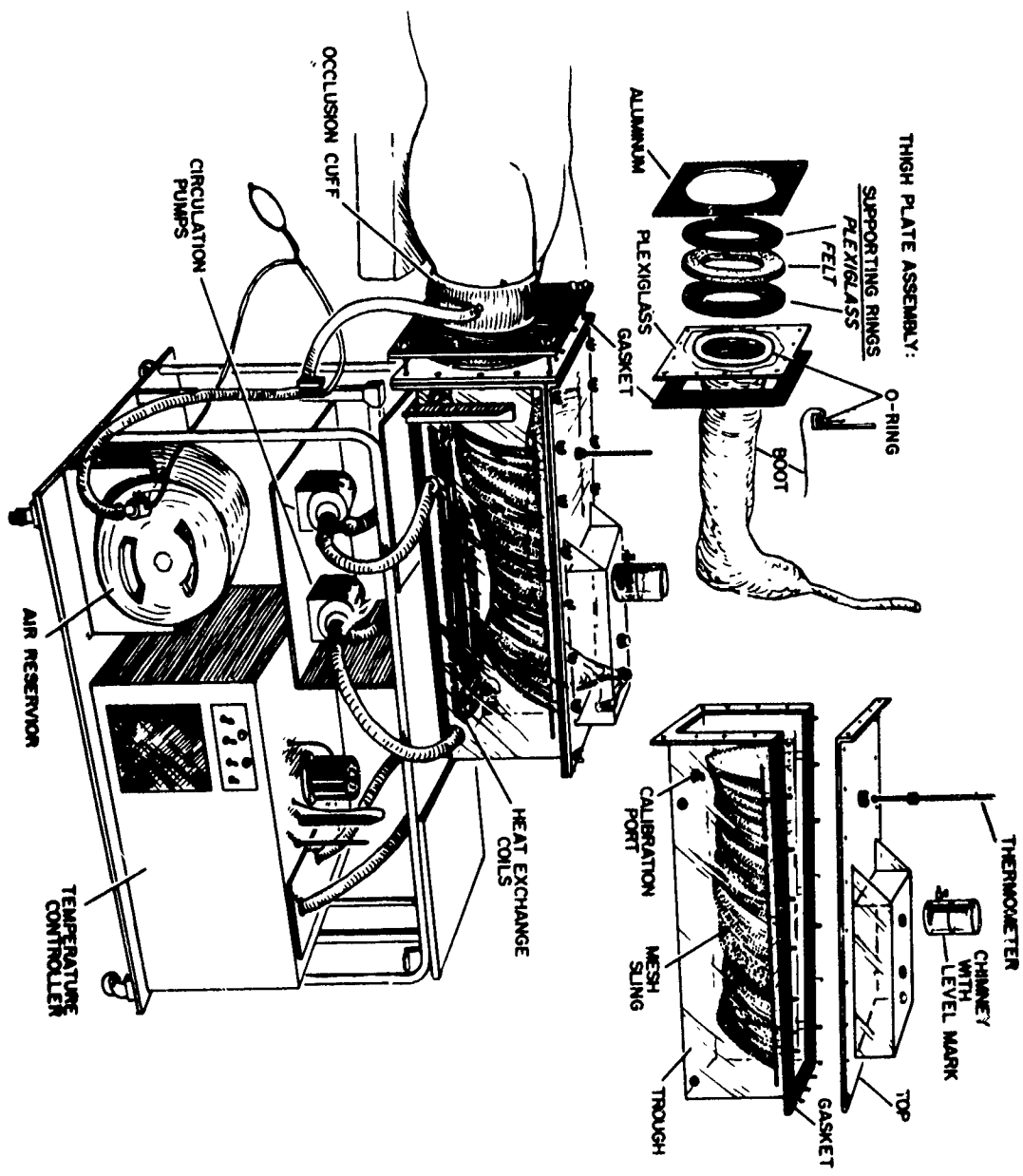


Figure 1. Leg plethysmograph.

a "chimney" (7.5 cm I.D.) which permits water displacement from the plethysmograph with limb swelling..

A loose-fitting, full length, boot of thin (0.05 mm) clear, polyvinyl sheeting was used to form a watertight seal around the leg when in the plethysmograph, preventing fluid exchange across the injured limb and minimizing contamination of the wound. A narrow tube of this same material is attached to the toe of this boot. The tube is passed through a small port in the top of the plethysmograph to permit air, caught between the skin and the boot, to escape as water is added. As soon as the boot is fully collapsed, the exit port is plugged with the connecting tube in place.

A circular cuff of polyvinyl sheeting at the proximal end of the boot serves to attach the boot to the thigh plate of the plethysmograph. Before the leg is placed into this boot, the proximal cuff is pulled through the opening of the thigh plate, stretched tight over a raised, grooved lip of this opening, and held in place by an O-ring. To minimize bulging of this flexible dam, the cuff is backed by three supporting rings (one 12.8 mm felt ring sandwiched between two rings of 6.4 mm plexiglass). A set of rings of various dimensions was prepared to accommodate limbs of different sizes. The supporting rings are held in place by a 6.4 mm aluminum plate bolted to the thigh plate.

The leg is slipped into the boot with the thigh plate and supporting rings attached. The thigh plate is advanced up the leg until the felt supporting ring forms a close, nonconstricting cuff around the upper thigh (approximately 15-20 cm distal to the symphysis pubis). The leg is then placed into a nylon mesh sling, and the three sections of the plethysmograph are locked into place. Throughout the rest of the study, the subject rests supine with the leg abducted about 30° at the hip and at the level of or slightly above the heart.

Water is added through a port in the bottom of the plethysmograph until it reaches a set level in the chimney. In order to standardize hydrostatic pressure across the limb, this same level is re-established prior to each blood flow determination. Water temperature is maintained at the mean leg skin temperature by a Forma refrigerated and heated bath and circulator, which circulates ethylene glycol at a set temperature through copper tubing located in the floor of the plethysmograph. The water is continuously stirred by two small centrifugal pumps.

Venous occlusion is accomplished by rapidly inflating a 10 cm wide tourniquet cuff placed on the exposed thigh. Inflation pressure is created by filling a large air reservoir located beneath

the plethysmograph. In some patients with burns on the thigh, a topical anesthetic (2% viscous lidocaine) was applied to the area under the tourniquet cuff to reduce pain resulting from inflation. The distance between the cuff and the plethysmograph is minimized but may be adjusted in order to distinguish the inflation artifact from actual limb volume changes. Venous occlusion pressures were varied in each experiment to maximize the rate of limb swelling. The usual occlusive pressure ranged from 80-140 mmHg.

Change in limb volume with venous occlusion causes water to rise in the chimney. The associated increase in column hydrostatic pressure is converted to an electrical signal (Statham Model P23BB pressure transducer), amplified (Sanborn 964 recorder), and recorded on a Leeds and Northrup Model 620 recorder.

Using this recording system, the inflation artifact is readily identifiable and lasts 2-3 seconds following limb occlusion. Leg volume changes between 4 and 10 seconds were used to determine leg blood flow. In the vast majority of measurements, this portion of the curve was linear; but, when this segment was curvilinear, the slope between 4-6 seconds of occlusion was read.

The plethysmograph is calibrated with the leg in place prior to the experiment and after each two or three blood flow measurements. This is accomplished by injecting known volumes of water through a three-way stopcock located on one side of the chamber.

At the end of each study, the volume of water drained from the plethysmograph is measured. Limb volume is calculated by subtracting the measured volume plus the volume of the boot from the known capacity of the plethysmograph and leg blood flow is expressed per 100 ml of leg volume. The 8-10 flow measurements are averaged to determine mean resting leg blood flow.

This plethysmograph was designed and constructed specifically for use in burn patients. The long leg model was chosen for several reasons. First, it provided for comfortable, full-length support of burned limbs in order to achieve resting blood flow measurements in this particular patient population. Second, by incorporating a major portion of the limb, the effect of variations in the extent of limb injury could be evaluated. Finally, a plethysmographic cuff was not applicable in burn limbs because variations in the wound location could affect limb expansion, thereby precluding the use of circumferential changes of one segment as being representative of volume alterations of the entire limb.

Body Temperature Measurement

Total body mean skin temperature (\bar{T}_{sk}) was calculated by appropriately weighting nine skin temperatures according to the estimated surface area being represented (8):

$$\begin{aligned}\bar{T}_{sk} = & 0.143 T \text{ anterior thigh} + 0.081 T \text{ posterior thigh} + \\ & 0.168 T \text{ lateral calf} + 0.105 T \text{ low back} + 0.140 T \text{ forearm} \\ & + 0.050 T \text{ dorsum of hand} + 0.315 T \text{ abdomen.}\end{aligned}$$

Mean leg skin temperature (\bar{T}_{skl}) was calculated in a similar manner from five leg skin temperatures:

$$\begin{aligned}\bar{T}_{skl} = & 0.365 T \text{ anterior thigh} + 0.122 T \text{ posterior thigh} \\ & + 0.250 T \text{ lateral calf} + 0.083 T \text{ posterior calf} + 0.179 \\ & T \text{ foot.}\end{aligned}$$

RESULTS

Forty-five separate studies of resting leg blood flow and simultaneous measurements of rectal and surface temperatures were performed in the nine control subjects and 28 burn patients (Table 1). With an increase in the size of total body surface injury, there was a general increase in peripheral blood flow, central and surface temperatures and per cent leg burn (Table 2). The increase in leg blood flow was poorly related to total body surface burn ($r^2 = 0.33$) and rectal temperature ($r^2 = 0.27$), suggesting that the extra leg blood flow was not a manifestation of a generalized systemic response to injury.

Because some patients with burns as great as 50% of the total body surface had uninjured legs, the local influence of the wound on peripheral circulation was assessed by comparing a group of patients with burned legs with another group having comparable total body surface injuries but no burn on the leg under study (Table 3). The systemic response to injury was essentially the same in both groups of patients, as reflected by comparable rectal and total body mean skin temperatures. Leg blood flow and mean leg skin temperatures, however, were significantly increased by the local presence of the burn wound. Conversely, leg blood flow in the uninjured limbs of burned patients was similar to that of the normal controls.

8. Mitchell D, Wyndham CH: Comparison of weighting formulas for calculating mean skin temperature. J Appl Physiol 26:616-622, 1969.

TABLE 1. Physical characteristics of subjects, extent of burn injury, body temperatures, and leg blood flow

Subject	Age (years)	Sex	Weight* (kg)	PBD Studied	Burn Size		Temperatures (°C)			Leg Blood Flow** (ml/100 ml·min)
					% TBS	% Burn on Leg Studied	Rectal (T _{re})	Mean Skin (T _{sk})	Mean Leg Skin (T _{skL})	
Controls										
1a	24	M	75.0		0	0	37.2	34.2	34.1	3.29 ± 0.09
1b					0	0	36.9	34.4	33.9	3.12 ± 0.06
1c					0	0	37.0	34.7	33.7	2.97 ± 0.17
2	29	M	82.7		0	0	36.5	34.0	33.7	3.45 ± 0.10
3	35	M	104.5		0	0	37.1	34.7	33.9	2.30 ± 0.07
4	33	M	79.5		0	0	37.1	34.7	33.8	2.41 ± 0.06
5	35	M	79.5		0	0	37.1	34.3	32.6	2.02 ± 0.05
6	28	M	63.6		0	0	36.9	34.2	34.0	3.06 ± 0.24
7	23	M	71.1		0	0	36.9	34.0	33.6	1.73 ± 0.16
8	23	F	51.4		0	0	37.1	35.1	34.8	2.83 ± 0.20
9	22	F	65.9		0	0	37.3	35.0	34.5	2.91 ± 0.18

TABLE 1. Physical characteristics of subjects, extent of burn injury, body temperatures, and leg blood flow

Subject	Age (years)	Sex	Weight* (kg)	PBD Studied	Burn Size		Temperatures (°C)			Leg Blood Flow** (ml/100 ml·min)
					% TBS	% Burn on Leg Studied	Rectal (T _{re})	Mean Skin (T _{sk})	Mean Leg Skin (T _{skL})	
Burn Patients										
1	20	M	77.4	12	3	0	37.1	35.0	34.4	2.87 ± 0.10
2a***	15	M	54.5	7	6.5	0	37.4	35.2	35.0	3.24 ± 0.17
2b				7	6.5	32.5	37.4	35.3	35.7	6.52 ± 0.13
3	19	M	84.0	6	10	12	37.9	35.6	35.5	4.91 ± 0.11
4	36	M	80.8	10	12	0	37.9	34.9	33.8	2.61 ± 0.18
5	36	M	93.1	12	17.5	0	37.6	35.2	34.2	2.53 ± 0.10
6	18	M	65.7	10	18	2.5	37.8	34.7	34.6	2.56 ± 0.08
7	25	M	52.3	18	19.5	45	38.0	36.1	35.1	5.27 ± 0.29
8	40	M	83.0	7	23	0	37.6	34.5	33.2	1.77 ± 0.07
9a	29	M	83.5	11	24.5	20	38.7	36.6	36.1	8.39 ± 0.27
9b				18	24.5	20	37.7	35.8	35.2	7.05 ± 0.07

TABLE 1. Physical characteristics of subjects, extent of burn injury, body temperatures, and leg blood flow

Subject	Age (years)	Sex	Weight* (kg)	PBD Studied	Burn Size		Temperatures (°C)			Leg Blood Flow** (ml/100 ml·min)
					% TBS	% Burn on Leg Studied	Rectal (T _{re})	Mean Skin (T _{sk})	Mean Leg Skin (T _{skL})	
10	16	M	62.0	7	25.5	0	38.2	37.0	35.8	3.07 ± 0.14
11a	20	M	83.7	7	28	70	38.6	36.2	35.9	7.48 ± 0.19
11b				21	28	70	39.1	36.8	36.9	11.73 ± 0.82
12	18	M	69.6	11	35	10	37.8	35.7	34.2	3.50 ± 0.13
13	33	F	71.8	13	39	0	38.7	35.4	34.5	3.43 ± 0.07
14a	22	M	61.8	7	41.5	45	38.8	35.9	35.0	5.88 ± 0.20
14b				14	41.5	45	37.5	35.8	35.2	6.24 ± 0.16
15	33	M	63.4	9	43	45	38.2	36.3	36.4	8.60 ± 0.45
16a	55	M	62.6	15	45	0	37.0	34.6	33.9	3.95 ± 0.11
16b				21	45	0	38.2	34.7	34.1	4.20 ± 0.18
17	25	F	69.5	9	45	63	37.8	36.2	35.4	7.59 ± 0.25
18	40	M	60.0	12	45	17.5	38.1	35.7	33.9	4.04 ± 0.06
19	19	M	72.8	12	48	12.5	39.6	37.2	35.7	4.48 ± 0.11

TABLE 1. Physical characteristics of subjects, extent of burn injury, body temperatures, and leg blood flow

Subject	Age (years)	Sex	Weight* (kg)	PBD Studied	Burn Size		Temperatures (°C)			Leg Blood Flow** (ml/100 ml·min)
					% TBS Studied	% Burn on Leg Studied	Rectal (T _{re})	Mean Skin (T _{sk})	Mean Leg Skin (T _{skL})	
20	19	F	73.1	9	48	63.0	38.1	36.4	36.7	8.54 ± 0.11
21a	26	M	73.2	10	50	0	38.9	36.7	36.1	2.99 ± 0.21
21b				17	50	0	38.3	36.0	34.8	3.17 ± 0.41
22	28	M	77.2	21	50	52.5	38.3	36.3	35.7	6.24 ± 0.24
23	20	M	59.1	10	50.5	37.5	38.7	36.3	35.9	6.36 ± 0.09
24	19	M	68.2	21	61.5	60	39.6	36.5	35.2	9.24 ± 0.42
25	22	M	81.6	7	74	77.5	38.9	37.2	36.8	7.75 ± 0.15
26	24	M	61.1	11	78	80	38.7	36.5	35.9	8.33 ± 0.17
27	28	M	58.4	20	82	87.5	36.3	33.2	33.2	7.51 ± 0.13
28	22	M	79.5	8	86	82.5	37.5	33.8	31.6	5.92 ± 0.25

*Body weights on initial study.

**Mean ± S.E.

***Patient had a burned (2b) and unburned (2a) leg.

TABLE 2. General effects of total body burn size on body temperatures and leg blood flow (mean \pm S.E. or range)

Burn Size	Controls	<25%	25-49%	>49%
No. of Subjects	9	9	11	8
No. of Studies	11	11	14	9
Age (Years)	28 (22-35)	26 (15-40)	27 (16-55)	24 (19-28)
Weight (kg)	74.8 (51.4-104.5)	74.9 (52.3-93.1)	70.8 (61.8-88)	69.8 (58.4-81.6)
% Total Body Surface Burn	0	15.0 (3-24.5)	40.5 (25.5-48)	66.5 (50-86)
% Leg Surface Burn	0	12.0 (0-45)	29.5 (0-70)	59.5 (0-87.5)
Postburn Day Studied	—	11 (6-18)	12 (7-21)	14 (7-21)
<u>Temperatures ($^{\circ}$C)</u>				
Rectal	37.0 \pm 0.1	37.7 \pm 0.1	38.3 \pm 0.2	38.4 \pm 0.3
Mean Skin	34.5 \pm 0.1	35.4 \pm 0.2	36.0 \pm 0.2	35.8 \pm 0.5
Mean Leg Skin	33.9 \pm 0.3	34.8 \pm 0.3	35.3 \pm 0.3	35.0 \pm 0.6
<u>Leg Blood Flow (ml/100 ml\cdotmin)</u>				
	2.74 \pm 0.16	4.34 \pm 0.67	5.91 \pm 0.69	6.39 \pm 0.72

TABLE 3. Local effects of leg burn wound on limb blood flow and surface temperatures (mean \pm S.E. or range)

	Controls	No Leg Burn*	Leg Burn**
No. of Subjects	9	8	8
No. of Studies	11	10	9
Age (Years)	28 (22-35)	30 (15-40)	23 (15-33)
Weight (kg)	74.8 (51.0-104.5)	70.7 (54.5-83)	68.8 (57.3-84)
% Total Body Surface Burn	0	25.5 (3-50)	26.0 (6.5-50)
% Leg Surface Burn	0	0	27.5 (2.5-52.5)
Postburn Day Studied	—	12 (7-21)	12 (6-21)
<u>Temperatures (°C)</u>			
Rectal	37.0 \pm 0.1	37.9 \pm 0.2	38.0 \pm 0.1
Mean Skin	34.5 \pm 0.1	35.4 \pm 0.3	35.8 \pm 0.2
Mean Leg Skin	33.9 \pm 0.2	34.6 \pm 0.3	35.4 \pm 0.2 ⁺
<u>Leg Blood Flow (ml/100 ml·min)</u>			
	2.74 \pm 0.16	3.13 \pm 0.21	5.89 \pm 0.68 ⁺

*Patients No. 1, 2a, 4, 8, 10, 13, 16a, 16b, 21a, 21b

**Patients No. 2b, 3, 6, 7, 9a, 9b, 12, 15, 22

⁺Unpaired t-test with patients without leg burn, $p < .05$

In all subjects studied, leg blood flow increased with the extent of the leg burn (Fig. 2). The relationship appeared curvilinear: limb blood flow approaching 8.0 ml/100 ml·min as the size of the leg burn exceeded 60%.

Increasing leg surface temperature by raising bath temperature 5°C increased limb blood flow to the same extent in patients with burned and unburned extremities as it did in the normal controls (Fig. 3). This circulatory response in the burned limbs was not solely determined by vasodilatation of the uninjured skin since comparable changes in leg blood flow occurred in patients with leg burns as great as 85% of the leg surface.

DISCUSSION

Leg blood flow measurements in these patients clearly demonstrate that peripheral blood flow is increased during the hypermetabolic-hyperdynamic phase of thermal injury. Since this extra blood flow was closely related to the extent of local injury, and not found in the uninjured legs, the increased peripheral blood flows appear to be directed primarily to the burn wound. As observed in earlier total body measurements and confirmed by this study, elevated blood flow accounts for a higher mean leg skin temperature on burned limbs. This is most evident in the two groups of patients with and without leg burns but comparable core and total body mean skin temperatures (Table 3). Since these two groups maintained similar levels of body heat content, the higher skin temperatures on the burned legs are the result of higher superficial blood flows. Considering the increased evaporative cooling which occurs from the burn wound (9,4), the impact of increased superficial blood flow on maintaining an elevated surface temperature is even more impressive.

Additional observations in our laboratory support the concept of wound-directed peripheral blood flow. In third degree wounds, which are associated with superficial vascular thrombosis (10), leg blood flow is near control levels shortly after injury. Flow

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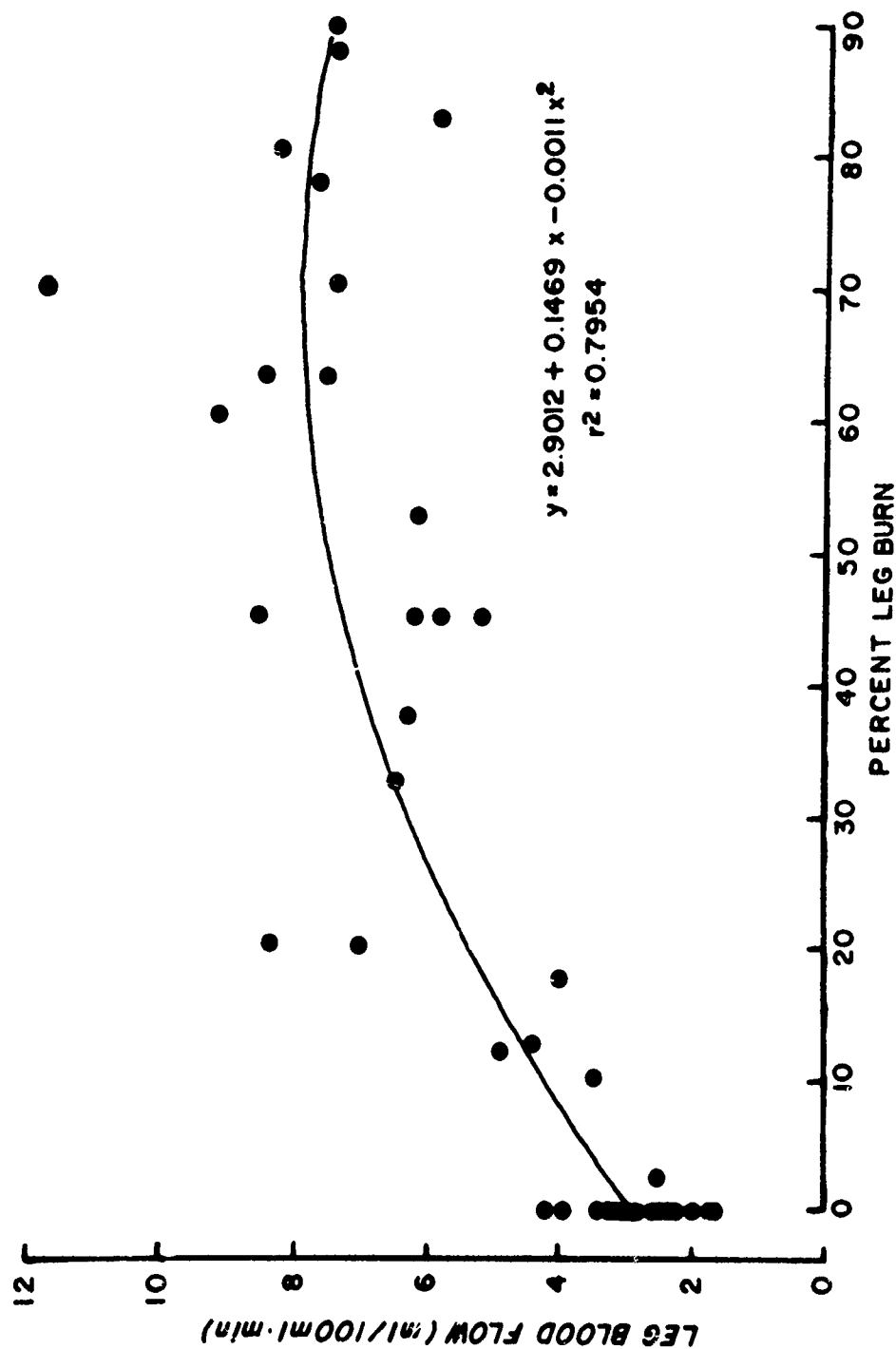


Figure 2. The relationship between leg blood flow and the size of the total leg surface injury. As approximately 90% of the leg surface was within the plethysmograph, this relationship was unaffected by expressing burn size as a per cent of leg surface within the plethysmograph.

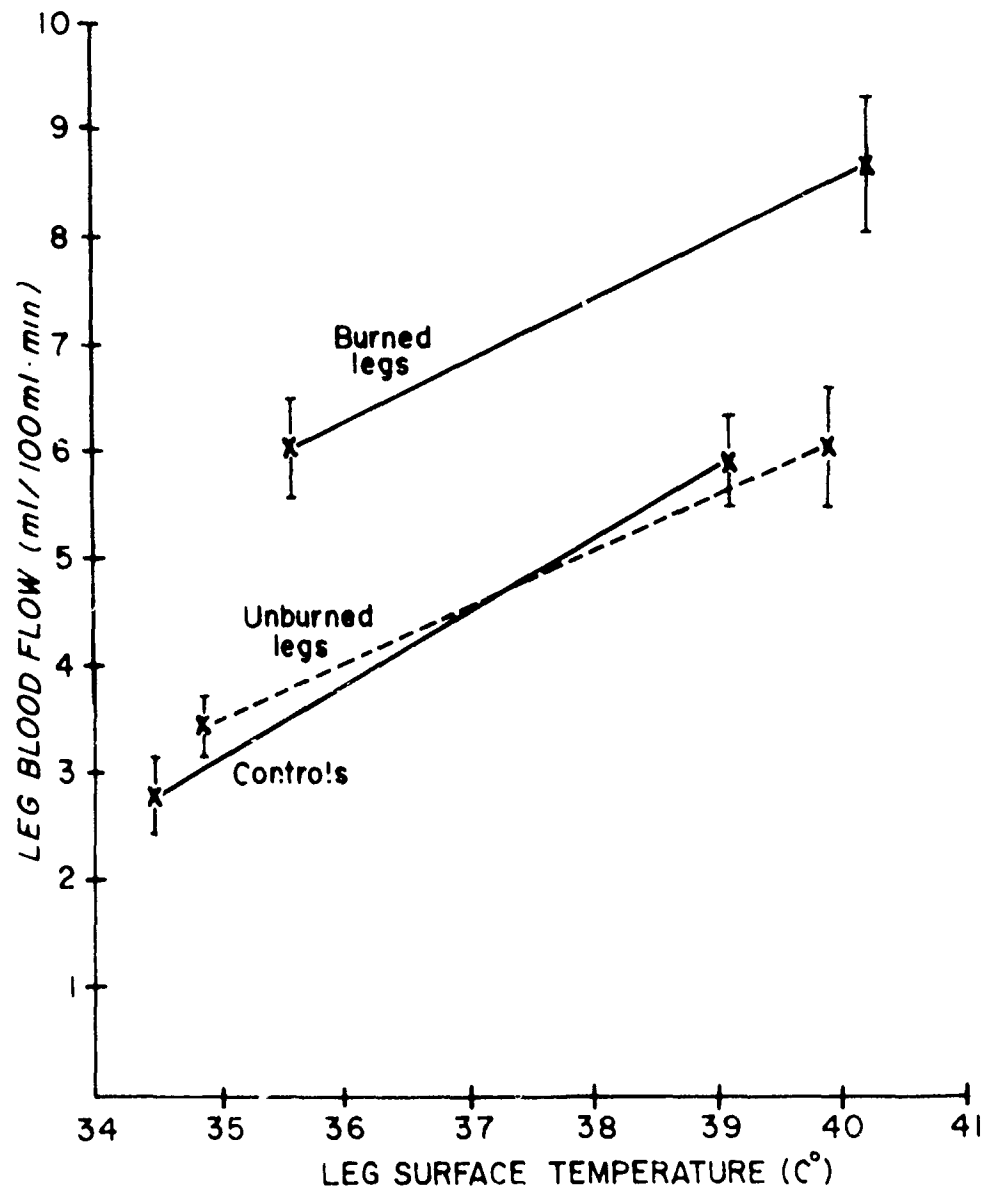


Figure 3. The effects of a 5°C increase in water temperature on leg blood flow in normal controls (subjects 1a, 2, 3, 4), patients without leg burns (patients 1, 10, 13, 16a, 16b), and patients with burned legs (patients 3, 6, 7, 14a, 14b, 20, 25, 27). While the flow to the burned legs is elevated, the response to leg heating was the same in all subjects. Each point represents the mean surface temperature and the mean and S.E. of the leg blood flow for each group.

increases to reach levels predictable from the size of leg burn by the end of the first week, associated with formation of a richly vascularized wound bed. In contrast, partial-thickness injury does not ablate the superficial vascular bed and blood flow is elevated in these legs as soon as circulatory volume is restored. In addition, leg blood flow measurements were performed in patient no. 28 prior to and five days after excision to fascia of the 32.5 leg surface wound. Leg blood flow dropped from 5.27 ml/100 ml·min to 3.33 ml/100 ml·min with the removal of the burn tissue.

Other studies suggested that increased peripheral blood flow occurs in burned patients (3) and related the increase in total body core-to-skin heat conductance to burn size (4). Patients with large burns were characterized by diminished core-to-skin insulation. This study demonstrates that the increased peripheral blood flow and heat conductance previously reported are confined to the burn wound. Patients with small burns have less of an insulative deficit, and, consequently, can store heat more effectively than extensively injured patients. Severely burned individuals usually do not increase body temperature in proportion to burn size and often maintain cooler skin and wound surface temperatures than patients with smaller burns. Since the insulative deficit increases with burn size, but the rate of metabolic heat production reaches maximal levels (approximately twice normal) in patients with burns greater than 60% TBS injuries (11), the more extensively injured patients are extremely vulnerable to hypothermia when exposed to cool environments.

The plateau or upper limit of leg blood flow to the more extensively burned limbs does not reflect maximal vasodilation of the wound, since blood flow to these legs increased when the temperature of the surface was raised 5°C. Vascular smooth muscle tone in the granulating wound is therefore, at least, sensitive to changes in local temperature, but the additional influences of other local metabolic factors and/or central reflex activity on wound perfusion remain to be identified. Since peripheral or wound blood flow is affected by variations in surface temperature, an elevation in ambient temperature should permit the burned patient to increase wound blood flow and presumably improve healing.

3. Gump FE, Price JB Jr, Kinney JM: Blood flow and oxygen consumption in patients with severe burns. Surg Gynec Obstet 120: 23-28, 1970.

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11. Wilmore DW, Long JM, Mason AD Jr, Skreen RW, Pruitt BA Jr: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-669, 1974.

In summary, peripheral blood flow measurements in 28 burn patients revealed that leg blood flow was increased only to the injured limbs. Leg blood flow was closely related to the extent or size of leg burn, indicating that most of the increased peripheral blood flow was directed to the burn wound. Variations in surface temperatures modified this peripheral vascular response to injury. The dominance of local wound effects on the distribution of peripheral blood flow in patients with marked systemic alterations in metabolism and thermoregulation demonstrates the priority of the wound in the body's homeostatic response to injury.

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PRESENTATIONS AND/OR PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^b	REPORT CONTROL SYMBOL	
				DA OG 6966	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^c	6. WORK SECURITY ^d	7. REGRADING ^e	8. DISEM INSTR ^f	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF DOW
76 10 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO /CODES ^g	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62110A	3A162110A821		00	107		
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^h							
(U) Intrarenal Blood Distribution: A Non-invasive Technique For Determining The Intrarenal Distribution of Blood Flow in a Model Simulating Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁱ							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 11		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				PRECEDING		.5	
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d. KIND OF AWARD.				COUNTRY			
e. AMOUNT:							
f. CUM. AMT.							
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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ADDRESS ^k Fort Sam Houston, Texas 78234				ADDRESS ^k Burn Study Branch Fort Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: William D. Myers, LTC, MC			
				NAME: Harry R. Jacobson, MAJ, MC			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Intrarenal blood distribution; (U) DMSA; (U) Renal scan; (U) Dogs							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number Precede text of each with Security Classification Code)							
23. (U) To determine a non-invasive technique for evaluating intrarenal distribution of blood flow in a laboratory animal model simulating the burned soldier.							
24. (U) Animals will be anesthetized. A carotid artery cannula will be placed as well as a jugular cannula. Dimercapto succinic acid will be infused intravenously and the kidneys will be scanned. Following the termination of the scanning labelled microspheres will be injected intraarterially. The animal will then be sacrificed, the kidneys harvested and the exact renal distribution of blood flow will be determined by counting the distribution of injected microspheres. A correlation between the non-invasive scan technique and actual renal blood flow distribution as determined by the microspheres will be sought.							
25. (U) 75 11 - 76 09 Fourteen rats have had the right ureter ligated for periods of 2 days to 3 months in an attempt to alter renal blood flow. The contralateral kidney was used as a control. Preliminary studies indicate that using a graphical analysis of Dimercaptosuccinic acid uptake by the normal kidney a ratio of 1.73 for cortical to cortical medullary blood flow is obtained. A ratio of 1.75 was obtained by analysis of microsphere distribution. Obstructed kidneys have also been studied but too little data is as yet available for a meaningful analysis. Other means of altering blood flow are also being sought to confirm this method.							

*Available to contractors upon originator's approval

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1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 68
AND 1498 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

TERMINATION

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

**REPORT TITLE: INTRARENAL BLOOD DISTRIBUTION: A NONINVASIVE TECHNIQUE
FOR DETERMINING THE INTRARENAL DISTRIBUTION OF BLOOD
FLOW**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234
1 July 1975 - 30 September 1976**

Investigators:

**W. Scott McDougal, M.D., Major, MC
William D. Myers, M.D., Lieutenant Colonel, MC
Robert J. McAuley, M.S., Captain, MSC
Robert Lull, M.D., Lieutenant Colonel, MC**

Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: INTRARENAL BLOOD DISTRIBUTION: A NONINVASIVE TECHNIQUE
FOR DETERMINING THE INTRARENAL DISTRIBUTION OF BLOOD
FLOW

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 July 1975 - 30 September 1976

Investigators: W. Scott McDougal, M.D., Major, MC
William D. Myers, M.D., Lieutenant Colonel, MC
Robert J. McAuley, M.S., Captain, MSC
Robert Lull, M.D., Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

The intrarenal distribution of blood flow was determined by a new noninvasive technique. Technetium labeled dimercapto-succinic acid was injected intravenously and timed retention by the kidney recorded. Graphical analysis of the uptake curve provided values for two zones of blood flow and compared favorably to values for the cortex and juxta-medullary region as determined by microsphere injection. When the distribution of blood flow was altered by ureteral ligation, the graphical values compared favorably with those obtained by microsphere analysis.

Renal Blood Flow
Dimercaptosuccinic Acid
Differential Renal Blood Flow

A NONINVASIVE TECHNIQUE FOR DETERMINING THE INTRARENAL DISTRIBUTION OF BLOOD FLOW

A curious form of renal insufficiency is periodically observed in the thermally injured patient. Glomerular filtration rate is diminished, but unlike classic acute renal failure oliguria does not occur and urinary sodium excretion is exceedingly small. The syndrome is heralded by laboratory evidence of prerenal failure in the absence of recognizable clinical causes. The avidity of the nephrons for sodium, their continued ability to secrete potassium, and the early prerenal character of the syndrome might be explained by a shift in the intrarenal blood flow from the cortex to the medulla. Thus, the nephrons with the long loops of Henle which are known to have an increased capacity for sodium reabsorption as well as urea retention are maximally perfused. The cortical nephrons would contribute little to the formation of urine, thus explaining the observed abnormalities. Supportive evidence for this hypothesis has been gained from the experimental animal in which a redistribution of renal blood flow has been demonstrated in the early postburn period.¹

Two methods are currently available for determining the distribution of renal blood flow. The first involves an intra-arterial injection of labeled microspheres followed by section of the kidney, isolation of the various zones and counting the microspheres in the tissue specimens. This technique obviously cannot be applied to patients. The second method utilizes a radioactive nuclide of an inert gas, either xenon or krypton, and involves injection of the nuclide directly into the renal artery followed by recording the disappearance of radioactivity from the kidney. The disappearance curve is stripped into four components, the first three representing specific zones of blood flow.^{2,3,4} The method is cumbersome, requires manipulation of an arterial cannula and does not lend itself to studying acutely ill patients who demand continuous intensive care monitoring. Therefore, if the proposed mechanism of acute renal failure in the thermally injured patient is to be substantiated, a more practical approach must be designed. The distribution of blood flow determined by a nuclide injected intravenously followed by

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1. Carter JG, Wells CH: Intrarenal redistribution of blood flow in the early postburn period. *J Trauma* 15:877, 1975.
 2. Ladefoged J: Renal cortical blood flow and split function test in patients with hypertension and renal artery stenosis. *Acta Medica Scandinavica* 179:6, p 641, 1966.
 3. Ladefoged J, Pedersen F: Renal blood flow, circulation times and vascular volume in normal man measured by the intra-arterial injection - external counting technique. *Acta Physiol. Scand.* 69:220, 1967.
 4. Rosen SM, Hollenberg NK, Dealy JB, Merrill JP: Measurement of the distribution of blood flow in the human kidney using the intra-arterial injection of ¹³³Xe. Relationship to function in the normal and transplanted kidney. *Clin. Sci.* 34:287, 1968.

a renal scan and graphical analysis would obviate many of the difficulties and risks of the washout method and would lend itself quite well to the study of critically ill patients. The purpose of this study is to determine if the nuclide technetium labeled dimercapto-succinic acid (DMSA) given intravenously followed by renal scanning and graphical analysis is an accurate method of determining the intrarenal distribution of blood flow.

MATERIALS AND METHODS

Preparation of the experimental animals. Eight Sprague Dawley rats were anesthetized with sodium pentobarbital (0.025 mg per gm body weight), the abdomen entered through a lower midline incision and the right ureter ligated. The animals were allowed food and water ad lib. They were reanesthetized from two to sixty days postligation with sodium pentobarbital, a jugular and superior aortic arch cannula placed, and the animals positioned under a Searle Hp Pho-Gamma camera. One millicurie of DMSA (Medi-physics) was injected intrajugularly and both kidneys scanned at 30 second intervals over a 45-minute period. At the termination of the experiment, approximately 40,000 Strontium 85 (1.0 uc) labeled microspheres (3 M Company) were injected directly into the aorta and the animal sacrificed. The kidneys were removed, weighed and placed in glutaraldehyde. Twenty-four hours later each kidney was sagittally sectioned and the cortex, juxtamedullary and medullary zones dissected. Each tissue zone was weighed, the technetium allowed to decay and the strontium counted in a standard well scintillation counter (Chicago Nuclear).

Graphical analysis. The uptake curve conforms to the general equation $q = C + (A - C)e^{-t/k}$ where q is the value at time t , A , the value at time 0 and C the value of the asymptote.⁵ The curve obtained for the uptake of DMSA by the kidney is of the second order and therefore the general form of the equation may be expanded to $q = C + (A - C)e^{-t/k_a} + (B - C)e^{-t/k_b}$ where q is the amount of nuclide contained in the kidney at time t , A the quantity of nuclide present as a function of the first component at $t = 0$, B the quantity of nuclide as a function of the second component at $t = 0$ and C the asymptote.

Several requisites must be satisfied if the uptake equation is to be related to blood flow: 1) DMSA must achieve rapid equilibrium between the blood and the renal tissue, 2) Once taken up by the tissue, significant back diffusion into the blood must not occur, 3) there should be little excretion into the tubular lumen, and 4) the partition coefficient must be the same for all renal zones. Given these assumptions, as t approaches 0, the renal vein concentration of DMSA also approaches 0 and the arterial concentration is proportional to the blood flow. If the

5. Defares JG, Sneddon IN: An introduction to the mathematics of medicine and biology. Year Book Publishers, Chicago, 1960, pp. 554-552.

blood flow for specific areas differs from that in other regions, the equation will be greater than the first order and the concentration of nuclide represented by each component will be proportional to the blood flow of a particular area. Therefore, from the expanded equation when $t = 0$, A and B represent the components of total isotope which would be retained if all the isotope injected were presented to the kidney simultaneously and instant equilibrium were achieved. Since the components are proportional to blood flow, the percentage distribution follows directly.

If the same assumptions regarding the rate constants for inert gas washout curves are made for the uptake curves, then flow per gram tissue for the zone represented by the rate constant may be calculated by the equation $K_n = F/W$, where K_n is the rate constant for component n, F the flow, W the weight of the tissue and the partition coefficient between tissue and blood for the indicator.⁶

RESULTS

The ratio of component A to component B determined by graphical analysis for seven normal kidneys was 1.73 ± 0.45 . The ratio of cortical to juxtamedullary blood flow determined by microsphere injection was 1.76 ± 0.59 for four normals and did not differ significantly from the ratio obtained by graphical analysis. Three ligated kidneys demonstrated a graphical ratio of 1.47 ± 0.23 which did not significantly differ from the two ligated kidneys analyzed by microspheres (1.58 ± 0.32). The values obtained for ligated kidneys did not significantly differ from those for normal kidneys in either the microsphere or graphical groups.

Rapid counting of three normal kidneys and bolus injection of the nuclide demonstrated a sharp increase in counts over the kidney to a maximum followed by a slight fall in the total count. As the bolus circulated and returned to the kidney, the sharp uptake followed by a moderate dip was repeated. Simultaneous measurement over the heart confirmed the bolus effect without dissipation (counts over the heart during the period when the bolus was elsewhere in the vascular system revealed values close to background). Since the peak kidney counts represent total isotope presented to the tissue and the troughs that which is retained, the ratio of the two gives the partition coefficient for DMSA for the entire kidney. Seven curves in three rats were analyzed giving a ratio of 0.76 ± 0.05 .

Timed bladder scans were obtained in 8 animals, each with at least one normal kidney, and demonstrated insignificant accumulation of activity during the first 10 to 15 minutes following injection. The ratio of renal activity to bladder activity at 15 minutes was 17:1. Counts of the vascular system, bladder, background, liver and kidney revealed almost

6. Ladefoged J: Measurements of the renal blood flow in man with the ¹³³Xenon washout technique. *Scandinav. J. Clin. & Lab. Investigation* 118:299, 1966.

exclusive accumulation in the latter.

DISCUSSION

Dimercapto-succinic acid labeled with technetium is rapidly taken up by the renal tissue as demonstrated by the analysis of a bolus of the agent passing through the kidney with the determination of the quantity retained and the partition coefficient. Retention by the kidney is demonstrated by the rapid fall off in radioactivity from the heart and lack of accumulation in the liver and muscular tissue. The bulk of nuclide remained in the kidney and initially, the bladder was free but after 30 minutes, a moderate amount was present. Thus, the agent satisfies the criteria of retention and insignificant dissipation during the first 10 to 15 minutes following injection. The uniformity of the partition coefficient throughout the kidney cannot be accepted with certainty since no experimental maneuvers were performed which would shed light on the question. Therefore, the criteria necessary of DMSA for determining regional renal blood flow from the uptake curve have for the most part been met.

Microsphere determination of regional blood flow is generally accepted as the most accurate method and the very close correlation of the microspheres to graphical analysis of DMSA uptake in the normal kidneys is encouraging. One method of altering renal blood flow is by obstructing the kidney.⁷ Although the correlation between injected microspheres and DMSA analysis for the obstructed kidneys was quite good, very little alteration in blood flow was obtained with ligation.

Although promising, confirmation of the usefulness of DMSA analysis for regional renal blood flow determination must come from correlations with microspheres in animals who have significant alterations in regional perfusion. Such studies are currently in progress.

PRESENTATIONS AND/OR PUBLICATIONS

None

7. Bay WH, Stein JH, Rector JB, Osgood RW, Ferris TF; Redistribution of renal cortical blood flow during elevated ureteral pressure. Am J Physiol. 222:33, 1972.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL
				DA OG 6965	76 10 01	DD-DR&E(AR)636
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8a. DDB'S INSTR ^a	8b. SPECIFIC DATA- CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
76 10 01	H. TERM	U	U	NA	NL	
10. NO / CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER
		62110A		3A162110A821		00
a. PRIMARY						115
b. CONTRIBUTING						
c. CONTRIBUTING						
11. TITLE (Precede with Security Classification Code) ^a (U) Glucagon and Heat Production in Normal Man and Septic Burn Patients in a Military Population (44)						
12. SCIENTIFIC AND TECHNOLOGICAL AREA ^a 003500 Clinical Medicine						
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD
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e. AMOUNT:						9
f. CUM. AMT.						
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION		
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research		
ADDRESS: Fort Sam Houston, Texas 78234				Surgical Study Branch		
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER		
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS		
				Louis H. Aulick, MAJ, MC		
				AA: Arthur D. Mason, Jr., M.D. DA		
22. KEYWORDS (Precede EACH with Security Classification Code)						
(U) Calorigenesis; (U) Gluconeogenesis; (U) Glucago: (U) Humans						
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)						
23. (U) To evaluate the contribution of hepatic gluconeogenesis to total body heat production in burned and burned infected soldiers.						
24. (U) Five normal men and four burn patients will be studied before and following the infusion of 2 mg glucagon for two hours. Oxygen consumption, core temperature, serum glucose, insulin, and glucagon will be measured serially throughout the study.						
25. (U) 75 07 - 76 09 Glucagon increased metabolic rate in both normals and burn patients. The effect appears dose related and occurs in time with the movement of glucose from the liver to the periphery. Glucose infusion does not duplicate this effect.						

^aAvailable to contractors upon originator's approval

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AND 1498-1 1 MAR 56 (FOR ARMY USE) ARE OBSOLETE

TERMINATION REPORT

project no. 3A162110A821-00, COMBAT SURGERY

**REPORT TITLE: GLUCAGON AND HEAT PRODUCTION IN NORMAL MAN AND SEPTIC
BURN PATIENTS IN A MILITARY POPULATION**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Louis H. Aulick, PhD, Major, MSC
Douglas W. Wilmore, MD
Arthur D. Mason, Jr., MD**

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: GLUCAGON AND HEAT PRODUCTION IN NORMAL MAN AND SEPTIC BURN PATIENTS IN A MILITARY POPULATION

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Investigators: Howard L. Aulick, PhD, Major, MSC
Douglas W. Wilmore, MD
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

Metabolic rate, urinary catecholamines, serum glucose, and insulin were determined serially in 23 studies in six normal men during two hours of rest followed by two hours of intravenous glucagon infusion. Glucagon doses ranged from 0-2 mg/hr. Metabolic rate increased 20% above resting levels. This relationship was: metabolic rate, kcal/m²/hr = 42.2, dose mg/hr 0.055, r² = 0.758, p < 0.001. Glucagon calorogenesis was unrelated to urinary catecholamine excretion rate and unaffected by beta adrenergic blockade in three subjects. Glucose, insulin, and respiratory quotient increased with glucagon administration, indicating augmented mass flow of glucose from the liver to the periphery. Glucagon calorogenesis was not related to increased cellular uptake of glucose: glucose infusion in six studies produced similar hyperglycemia and insulin responses but failed to produce comparable hypermetabolism. Glucagon infusion increased heat production, core temperature, and blood glucose in four septic burn patients. Glucagon calorogenesis is associated with hepatic glucose production and these effects are not mediated by the beta adrenergic nervous system.

Glucagon
Hypermetabolism
Glucose flow

GLUCAGON AND HEAT PRODUCTION IN NORMAL MAN AND SEPTIC BURN PATIENTS

Injury or infection in man increases the mass flow of glucose from the liver to peripheral tissues, and the rate of hepatic glucose production is related to the extent of injury and degree of hypermetabolism.¹ Gram negative bacteremia in burn patients decreases both mass flow of glucose and oxygen consumption.^{2,3} This study, describing the calorogenic response to glucagon, relates this response to other glucagon-mediated metabolic events and evaluates mechanisms which could mediate the increased heat production.

METHODS AND MATERIALS

Subjects

Six healthy normal males participated in this study, which was performed over a four-month period (Table 1). All subjects were active, without previous disease processes, and were well acquainted with metabolic and respiratory testing. The subjects maintained their customary dietary intake and activity status throughout the four-month period of investigation and were rarely studied more than once a week. The night before the study, the subjects did not eat after 7:00 P.M., but were allowed to drink water until midnight.

Procedure

The studies were performed in an environmental chamber, previously described, with ambient temperature maintained at 28°C and a relative humidity of 50 per cent. Subjects entered the room between 6:00 and 7:00 A.M. on the day of the study, changed to light cotton shorts, and rested in the recumbent position in bed. A probe of copper constantan was placed 8 cm into the rectum, and temperature was monitored at 10-minute intervals. Intravenous infusion needles were placed in large bore veins of one or both forearms for infusion and blood sampling, and patency was maintained by infusion pumps with 0.04 molar saline infusion at 60 ml/hr. After 30 to 60 minutes

1. Wilmore DW, Mason AD Jr, Pruitt BA Jr: Alterations in glucose kinetics following thermal injury. Surg Forum 25:81-83, 1975.
2. Wilmore DW, Mason AD Jr, Pruitt BA Jr: Altered glucose flow in septic burn patients. In press, Surg Gynec Obstet.
3. Wilmore DW, Long JA, Skreen R, Mason AD Jr, Pruitt BA Jr: Catecholamines: mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-668, 1974.

TABLE 1
CHARACTERISTICS OF SUBJECTS STUDIED

Subject	Age (Years)	Weight (kg)	BSA (m ²)	Basal Measurements (Mean \pm S.E.)				
				Serum Glucose (mg/100 ml)	Insulin (μ U/ml)	Metabolic Rate (kcal/m ² /hr)	V _O ₂ (L/Min)	Respiratory Quotient
1	21	57.1	1.61	86 \pm 3	10.6 \pm 1.3	36.1 \pm 0.6	0.197 \pm 0.003	0.88 \pm 0.01
2	37	74.5	2.00	91 \pm 1	7.2 \pm 1.4	34.3 \pm 0.3	0.236 \pm 0.002	0.84 \pm 0.01
3	34	78.0	2.05	88 \pm 1	7.6 \pm 0.5	35.1 \pm 0.2	0.246 \pm 0.002	0.84 \pm 0.01
4	26	88.6	2.18	83 \pm 3	7.0 \pm 2.7	36.5 \pm 0.3	0.274 \pm 0.001	0.82 \pm 0.02
5	24	76.8	2.00	87 \pm 1	8.2 \pm 0.7	36.8 \pm 0.5	0.257 \pm 0.004	0.79 \pm 0.01
6	25	79.1	1.93	86 \pm 1	8.9 \pm 0.3	35.8 \pm 0.3	0.240 \pm 0.003	0.80 \pm 0.02
Mean	28	76.0	1.96	87	8.2	35.8	0.242	0.83

of recovery following venopuncture, the subjects voided, the time was recorded and designated as the start of the experiment. In the first infusion study, the metabolic rate of the subject was determined hourly for four hours. The four-hour study period was divided into a two-hour control period, followed by a two-hour experimental period. At the end of the two-hour control period, one intravenous infusion was replaced with a freshly prepared solution of 0.04 molar saline containing glucagon, with doses administered ranging from 0.05 mg to 2 mg per hour, with the infusion rate maintained at 60 ml per hour. Analysis of the solution by specific glucagon antibody confirmed the glucagon concentrations in the infusate were within 10 per cent of the specified dose. Control studies were performed in all subjects with the saline infusion maintained throughout the four hours and no glucagon given. The order of the dose of glucagon infused and control studies were randomized in all subjects. In addition, three subjects received a glucose infusion during the experimental period to evaluate the effect of glucose administration on metabolic activity. Venous blood samples were drawn for serum glucose and insulin concentration at the end of each hour of the control period and at 30-minute intervals throughout the experimental period. All subjects voided at the end of the two-hour control and two-hour experimental periods, and the volume was measured and an aliquot acidified and frozen for catecholamine analysis.

A similar protocol was used to evaluate the effect of beta blockade on glucagon calorigenesis in three subjects. At the end of the two-hour control period, 5 mg of propranolol were administered intravenously as a loading dose, followed by a constant infusion of 0.08 mg propranolol per minute, which was given along with a steady infusion of 0.5 mg glucagon per hour. The efficacy of beta blockade was determined at the end of the experiment by the inability of subjects to respond to beta stimulation (2 ug isoproterenol IV) by increasing pulse rate, or demonstrating a rise in plasma free fatty acids.

Analytical Techniques

Gas exchange of oxygen and carbon dioxide was determined hourly by the open-circuit technique previously described.³ Two Douglas bags were collected over 10 minutes and the results of the two measurements averaged. Serum glucose, insulin and glucagon, and urinary catecholamines were determined by methods previously described.³

3. Wilmore DW, Long JA, Skreen R, Mason AD Jr, Pruitt BA Jr: Catecholamines: mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-668, 1974.

Patients Studied

Four individuals with gram negative bacteremia and hypothermia were infused with two mg glucagon per hour for a two-hour period. Core temperature was monitored in all patients by a rectal thermistor and metabolic rate and serum glucose were measured in three of the four individuals before and at the end of the study period.

RESULTS

Metabolic Rate

Basal metabolic rate was within 3 per cent of predicted metabolic rate for trained subjects.⁴ Variations in a Douglas bag measurement during the control period or during the saline infusion did not exceed more than 6 per cent for any single subject studied over the two-hour basal or four-hour control periods. Maximum study-to-study variation in basal metabolic rate in the same subject did not exceed 10 per cent for any single individual studied over the four-month period.

Basal metabolic rate increased in a curvilinear manner with increasing doses of glucagon (Fig. 1). This metabolic effect was first evident at the dose of 0.25 mg glucagon per hour. Metabolic rate plateaued at approximately 20 per cent above basal levels of heat production. No significant difference in glucagon calorogenesis was noted between the first and second hour following glucagon infusion. Oxygen consumption for the first hour during all glucagon infusions was 262 ± 8 ml/min and 263 ± 8 ml/min for the second hour. Mean respiratory exchange ratios (RQ) moved from 0.83 ± 0.01 during the basal period to 0.90 ± 0.01 during the first hour postinfusion, and 0.90 ± 0.01 during the second hour. No significant alterations were observed in rectal temperature at any level of glucagon infusion.

Altered Substrate Flow

Accompanying the alterations in metabolic rate were elevations in respiratory exchange ratio and serum insulin and glucose concentrations. Hyperglycemia and insulin response were highly variable. In general, the early elevation of serum glucose correlated positively with increasing glucagon doses, but the hyperglycemic response was masked by the stimulation of insulin with glucagon doses >0.5 mg/hr. Glucose infusion in an intermediate (12 g/hr) and large dose (36 g/hr)

4. Fleisch A: Le metabolisme basal standard et sa determination au moyen du "Metabocalculator," *Helv Med Acta* 18:23, 1951.

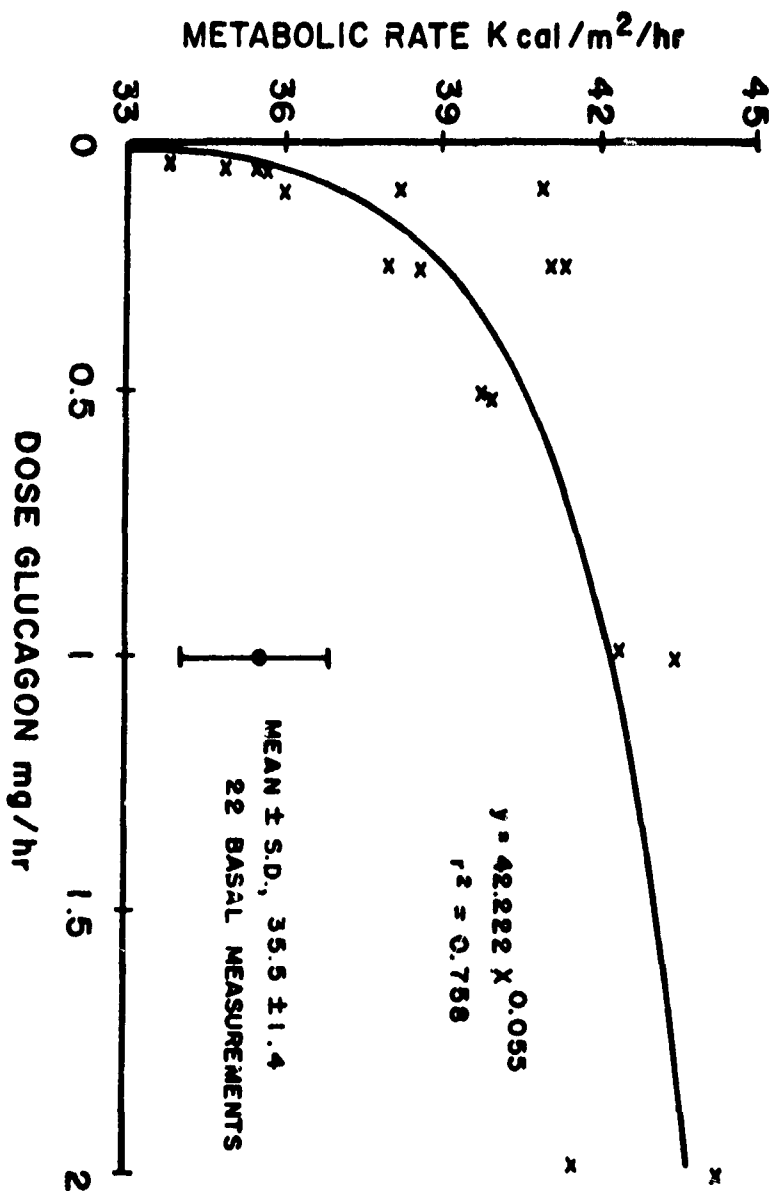


Figure 1. Metabolic rate increases in a curvilinear manner but does not exceed a 20% increase above basal (left). The dose response curve is observed when expressing per cent change in basal metabolism as a function of the log of the glucagon dose (right).

resulted in hyperglycemia and increased insulin elaboration, and was associated with an increase in respiratory exchange ratio. However, glucose infusion failed to produce comparable levels of heat production attained with glucagon doses which resulted in similar alterations in glucose and insulin concentration (Table 2).

Role of Catecholamines

Sympathetic activity, as quantitated by urinary excretion rate of catecholamines, was not related to glucagon calorigenesis. The mean excretion rate of urinary catecholamines was $5.6 \pm 0.6 \mu\text{g}$ per two hours during the control period, $6.7 \pm 0.8 \mu\text{g}$ per two hours during all periods of glucagon infusion, and $6.2 \pm 0.8 \mu\text{g}$ per two hours during the infusion of glucagon doses of 0.25 mg per hour or greater (doses associated with increased heat production). These differences were not significant. Propanolol administration in doses known to adequately block metabolic responses to beta adrenergic stimulation did not significantly alter increased calorigenesis, which occurred following the 0.5 mg per hour glucagon infusion. Similarly, alterations in glucose or insulin response were not observed by beta adrenergic blockade (Fig. 2).

Patient Studies

Core temperature increased with glucagon infusion in all patients (Table 3). This was associated with an increase in metabolic rate and a rise in blood glucose, similar to the alterations that were observed in normal individuals.

DISCUSSION

Glucagon calorigenesis has been described in both animals and man, although the exact mechanisms of the increased heat production were not known. In small animals, glucagon administration stimulates epinephrine elaboration, and it was suggested that the increased calorigenesis was mediated through the sympathetic nervous system.⁵ However, in this study, urinary catecholamines failed to show any elevation in this neurohormonal transmitter following glucagon administration, and heat production was unaffected with beta blockade. Tests of thyroid function have been performed following glucagon administration in man, and thyroid hormone is not increased following infusion

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TABLE 2

METABOLIC EFFECTS OF GLUCAGON ADMINISTRATION AND GLUCOSE INFUSION

	EXPERIMENTAL	
	CONTROL	EXPERIMENTAL
<u>Glucose infusion (36 g/hr), N = 2</u>		
Serum glucose	86 \pm 2	220 \pm 44
Insulin	6.3 \pm 0.9	15.8 \pm 1.8
Metabolic Rate	35.4 \pm 0.0	37.2 \pm 1.0
Respiratory Quotient	0.76 \pm 0.0	0.84 \pm 0.04
<u>Glucagon infusion (0.25 mg/hr), N = 4</u>		
Serum Glucose	93 \pm 2	155 \pm 16
Insulin	8.4 \pm 1.1	77.8 \pm 27.9
Metabolic Rate	35.9 \pm 0.7	39.2 \pm 1.5
Respiratory Quotient	0.82 \pm 0.02	0.90 \pm 0.04

THE EFFECT OF β BLOCKADE ON GLUCAGON STIMULATED HEAT PRODUCTION

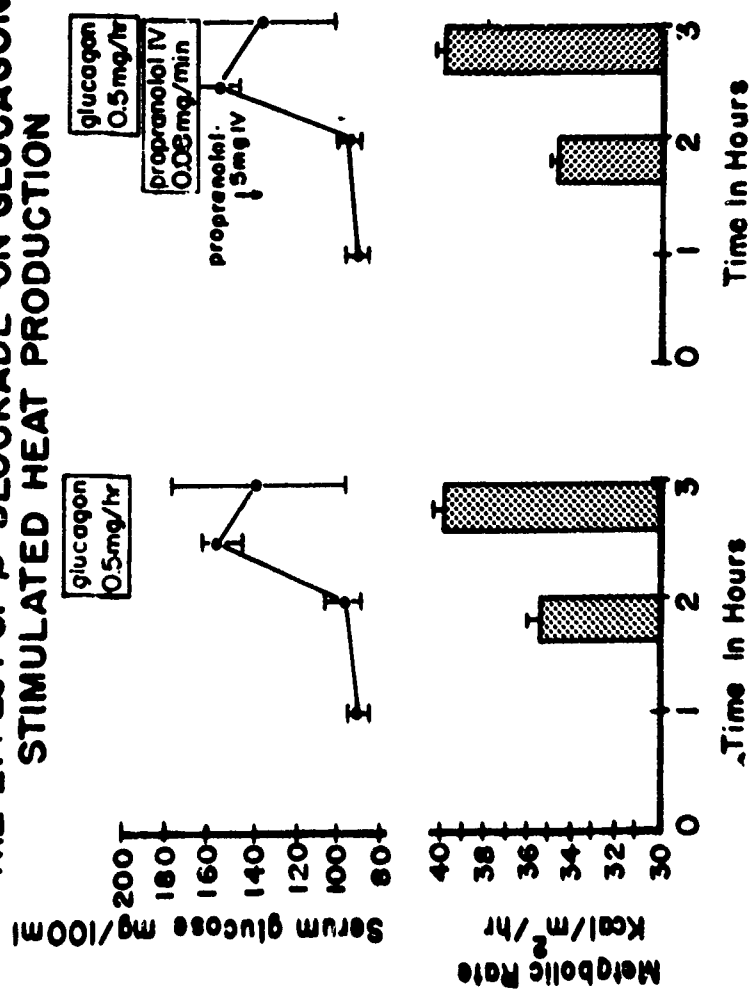


Figure 2. Beta adrenergic blockade did not affect the calorogenic or hyperglycemic response to glucagon.

TABLE 3
EFFECT OF TWO-HOURS OF GLUCAGON INFUSION GIVEN AT A DOSE OF 2 mg/HOUR

Patients	Age	% TBS Burn	P&D Studied	Metabolic Rate (kcal/m ² /hr)		Rectal Temperature (°C)		Blood Glucose (mg/100 ml)	
				Before	After	Before	After	Before	After
1	20	63	4	45.1	54.2	36.5	37.0	110	220
2	17	69	28	55.9	68.2	35.8	36.5	150	246
3	20	65.5	15	36.2	55.1	36.2	36.6	112	246
4	33	46.5	39	—	—	36.7	37.0	124	249

of this hormone.⁶ The possibility that the calorogenic effect was related to the mass flow of glucose into the peripheral tissues was also evaluated in this study. Although a slight increase in metabolic rate was noted during glucose infusion, the specific dynamic effect of glucose is small, and does not account for the total elevation of oxygen consumption which was observed in the normal subjects.

Glucagon acts specifically on the liver to enhance gluconeogenesis. Previous studies in severely injured patients reveal that the mass flow of glucose from liver to peripheral tissue is correlated closely with heat production, thus reflecting energy costs of gluconeogenesis. That heat production increases with the translocation of glucose from liver to peripheral tissue is observed by the increase in heat production which is accompanied by a rise in serum insulin and respiratory quotient. Diminished mass flow of glucose occurs in septic injured man, but heat production, core temperature, and mass glucose flow are increased and return toward pre-septic levels with the infusion of glucagon, thereby supporting the concept that the liver is the primary target organ which fails during gram negative endotoxemia. Alterations in circulation may affect liver function in septic man and glucagon may improve cardiac output, peripheral perfusion, and organ function in the patients studied. However, it is unlikely that increased support of splanchnic circulation alone accounted for the calorogenic effect of this hormone because a consistent rise in metabolic rate was observed in the normals following glucagon administration.

It should be noted that the maximum rates of heat production following a pharmacologic dose of glucagon infusion were relatively small. With infusion doses which mimicked portal glucagon levels observed in fasting man (0.05 mg/hr), no significant change in metabolic rate was observed. Burn patients have glucagon levels which are much higher than those observed in normal or fasting man,⁷ and this hormone may augment the heat production which occurs following activation of the adrenergic nervous system in injured man. The physiologic significance of this calorogenic effect of glucagon is yet to be defined.

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7. Wilmore DW, Lindsey CA, Moylan JA, Faloona GR, Pruitt BA Jr, Unger RH: Hyperglucagonemia after burns. Lancet 1:73-75, 1974.

PRESENTATIONS

Aulick LH. "Mechanisms of Glucagon Calorigenesis," presented at the American Physiological Society meeting (Federation of American Societies for Experimental Biology), Anaheim, California, 10 April 1976.

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Aulick LH, Wilmore DW, Mason AD Jr. Mechanisms of glucagon calorigenesis. Fed Proc 35:401, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	3. REPORT CONTROL SYMBOL	
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75 07 01	H. TERM	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO /CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62110A	3A162110A821		00		106	
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Aerosolized Gentamicin in The Prophylactic Treatment of Inhalation Injury in a Military Population (44)							
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ADDRESS: ^a Fort Sam Houston, Texas 78234				Burn Study Branch			
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				NAME: Hugh D. Peterson, COL, MC			
				NAME: Clement L. Slade, CPT, MC			
				DA			
23. KEYWORDS (Precede EACH with Security Classification Code) (U) Humans; (U) Inhalation injury; (U) Pneumonia; (U) Prophylaxis; (U) Gentamicin; (U) Aerosolized							
24. TECHNICAL OBJECTIVE: ^a 25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
<p>23. (U) The purpose of this experiment is to determine if aerosolized gentamicin is an effective agent in preventing pneumonia in burned soldiers who have sustained inhalation injury.</p> <p>24. (U) This will be a double-blind study. The patients will be divided into treatment and control groups. The groups will be subdivided by age and burn size. To enter the study the patient must have either a xenon scan or fiberoptic bronchoscopic evidence of inhalation injury. All patients will receive an aerosolized agent, either gentamicin 3.0mg/kg/24 hrs divided into 3 doses or normal saline on the same schedule. Therapy continues for 10 days following injury or until any pulmonary infiltrates have cleared. Leukens tube cultures will be taken on alternate days in an attempt to identify the emergence of resistant strains of bacteria.</p> <p>25. (U) 76 01 - 76 09 20 randomly selected patients have now entered the study. No conclusions can be drawn from this small group at this time. The code remains unbroken and it is not possible to identify two distinct groups on the basis of clinical course.</p>							

^a Available to contractors upon originator's approval

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1 MAR 66

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TERMINATION

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

**REPORT TITLE: AEROSOLIZED GENTAMICIN IN THE PROPHYLACTIC TREATMENT OF
INHALATION INJURY**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 July 1975 - 30 September 1976

Investigators:

**Barry A. Levine, MD, Major, MC
Hugh D. Peterson, DDS, MD, Colonel, MC
Robert B. Lindberg, PhD
Basil A. Pruitt, Jr., MD, Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A16211QA821-00, COMBAT SURGERY

REPORT TITLE: AEROSOLIZED GENTAMICIN IN THE PROPHYLACTIC TREATMENT OF INHALATION INJURY

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Basil A. Pruitt, Jr., MD, Colonel, MC

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Inhalation injury associated with cutaneous thermal injury, and the airborne pneumonia which frequently occurs as a complication, remain clinical problems for which there is no satisfactory treatment. Despite the use of various mechanical devices for pulmonary toilet and respiratory support, the prognosis for an inhalation injury associated with a large cutaneous thermal injury remains dismal. Although the early problems, e.g. laryngeal edema, bronchospasm, and pulmonary edema, can be treated, airborne pneumonia occurs with regularity and often with a fatal outcome.

It has been suggested that aerosolized antibiotics have a role in the treatment of airborne pneumonias. In various studies, aerosolized polymixin and gentamicin used prophylactically have been shown to reduce significantly the incidence of airborne pneumonia in certain groups of high risk patients. All observers have described the emergence of resistant strains of bacteria during such treatment.

This study's purpose is to investigate the effect of gentamicin, used prophylactically, on the course of inhalation injury. The questions asked are:

- 1) Is overall survival affected?
- 2) Is the incidence of airborne pneumonia affected?
- 3) Does the bacterial flora of the respiratory tract change and do resistant bacterial strains emerge?

This is a prospective, double blind study. The patients are divided into treatment and control groups. These groups are further subdivided by age and burn size. To enter the study, a patient must have evidence of inhalation injury proven by xenon scan and/or bronchoscopy. All patients receive an aerosolized agent, either gentamicin (3.0mg/kg/24

hours divided into 3 doses) or normal saline on the same schedule. Therapy continues for 10 days following injury or until pulmonary infiltrates have cleared. Luken's tube cultures of bronchial secretions are taken on alternate days in an attempt to identify the emergence of resistant strains of bacteria.

There have now been 20 patients randomized into the study. No conclusions can be drawn from so small a group and more patients will be studied before the code is broken and results analyzed.

Inhalation injury
Pneumonia
Prophylaxis
Gentamicin
Aerosolized

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